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PLANT ROOT EXCRETIONS IN RELATION TO THE RHIZOSPHERE EFFECT

III. THE EFFECT OF ROOT EXUDATE ON THE NUMBERS AND ACTIVITY OF MICRO-ORGANISMS IN SOIL

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INTRODUCTION

The very restricted nature of the rhizosphere with the major concentration of micro-organisms on the root surface has always indicated a zone of intense microbial activity, with its possible effect on the metabolism of the surrounding soil. Thom[14] suggested, without actual evidence, that the rhizosphere population played little or no part in the decomposition of the resistant soil organic matter, being capable only of attacking freshly added organic material.

The effects of the rhizosphere population on nitrification and on phosphate and manganese availability to plants have been investigated by other workers (Goring and Clark[6], Gerretsen[5], Timonin[15]). During a study of the microflora of roots of oat varieties, Timonin[15] found that varieties susceptible to manganese deficiency supported greater numbers of micro-organisms capable of oxidizing manganese into unavailable forms than did resistant varieties. Gerretsen[5] has shown that roots with a rhizosphere population were capable of utilizing insoluble mineral phosphates which were only slightly available to sterile roots. The recent work on the mineralization of nitrogen which has been reviewed by Clark[4] shows that crop growth generally suppresses mineralization. Goring and Clark[6]

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attribute this to the greater numbers of organisms in the soil surrounding the roots utilizing the soil nitrate during their growth and rendering it temporarily unavailable.

These investigations, however, have given no indication of the possible rôle of the rhizosphere population in the decomposition of organic matter in the soil apart from nitrogen. This is possibly due to the difficulty in dissociating the action of the roots from that of its surface flora.

In a study of the metabolism of rhizosphere soil, difficulty is experienced of obtaining sufficient soil, and hence it seemed desirable to develop a technique whereby large quantities of artificial "rhizosphere" soil could be produced. Timonin [15] has obtained a rhizosphere effect by burying colloidin sacs which contained flax root excretions but this method would not yield sufficient "rhizosphere" soil for the present investigation and the technique described below was developed. Using this technique it has been possible to study the changes in soil population and metabolism with root exudate supplements and the findings are reported in this paper.

EXPERIMENTAL

(*a*) *Establishment of an artificial rhizosphere*

Soils which had been air dried were brought to field capacity with root exudate solution (Rovira [12]), incubated in open vessels at 24°C and adjusted to field capacity daily with root exudate added solution. Treatment was continued for 14 to 21 days and the total exudate expressed as "plant equivalents per gramme of soil", each plant equivalent being the exudate from one plant during the first three weeks of growth. Parallel sets of soil treated with plant nutrient solution were used as controls.

(*b*) *Plate counts*

The medium of Bunt and Rovira [2] was used and the plates were incubated at 24°C for 14 days.

(*c*) *Qualitative changes*

The assessment of any qualitative changes in the populations due to treatment has been made on the basis of Gram staining and morphology. The selective stimulation of short Gram-negative rods appears to be one of the best established criteria for a rhizosphere effect, (Lochhead [7], Clark [3] and Rovira [10]), and in these experiments these qualitative

changes have been used as an index of the success or otherwise of the treatments.

(*d*) *Estimation of ammonia and nitrate*

Ammonia. This was estimated from 5% KCl extracts by treatment with alkali, distillation into 0.02*N* H_2SO_4 and titration of the residual acid.

Nitrate. After removal of the ammonia, the nitrate was reduced with Devarda's alloy, distilled into 0.02*N* H_2SO_4 and estimated as ammonia according to the method of Bengtsson[1].

(*e*) *Phosphate release from soil organic matter and nucleic acid*

The effects of root exudate on the phosphate level of the soil and the release from added nucleic acid were studied.

(i) Estimation of phosphate. The colorimetric method of Scheel[13] was used. Extraction was carried out with both 1% citric acid and 2% HCl.

(ii) Phosphate release from nucleic acid. The technique which Rogers[8] developed for the study of plant root exoenzymes in soil was followed. The sodium salt of yeast nucleic acid (B.D.H. preparation) was used, 5.13 g dissolved in 100 ml water and 10 ml ammonia (conc.), neutralised with H_2SO_4 and made up to 200 ml. This solution contains 2 mg P per ml. A suspension of 3 g of soil, 2 ml of nucleic acid solution and 4 ml of water was incubated at 28°C on a rotating shaking machine for 20 hours. The phosphate soluble in 1% citric acid and 2% HCl was then estimated.

(*f*) *Soil respiration*

The oxygen uptake of the soils subjected to the various treatments was measured at 30°C using the Warburg respirometer with 7 g of soil (wet weight) per flask (Rovira[9]). Duplicate flasks were always set up. The utilization of glucose by the soil population was studied by adding 1 ml of a 10% glucose solution per flask after the normal respiration had been recorded for three hours. The measurement of oxygen uptake was commenced 30 minutes after the addition of glucose to the soil.

RESULTS

(*a*) *Establishment of an artificial rhizosphere*

The effects of pea root exudate on the numbers of micro-organisms in a range of soils was studied, and as the results in Table I show there was a stimulation of the micro-organisms in all cases. The soils were selected because of different physical and chemical characteristics. Also included in Table I is the effect of oat root exudate on the population of Soil A.

TABLE I

The effect of root exudate supplements on the numbers of micro-organisms in various soils

Soil	Type	Number of bacteria *)/gramme ($\times 10^6$)		
		Control	Pea root exudate	Oat root exudate
A	Clay loam, crumb structure . .	70	166	186
B	Clay loam, poor structure . . .	65	218	—
C	Sandy loam, fair structure . .	57	91	—
D	Peat	183	453	—
E	Red loam, crumb structure . .	94	138	—

*) Means from four replicate treatments. S.E. = $\pm$ 7.8%.

The general increase of the bacterial population with root exudate supplements indicates the success in establishing an artificial rhizosphere. Fungal counts in treated soils showed no stimulation by the root exudate indicating an action similar to that in the root environment in which the bacteria are stimulated to a greater extent than the fungi.

Although the degree of stimulation (1.4 to 3.5) was not as great as often occurs in the rhizosphere, it is of a similar order to that found on the roots of three week old pea plants. Each gramme of soil had received an amount of exudate equivalent to that from 1.4 plants which is comparable to that in the normal rhizosphere where three week old pea plants growing in Soil B were found to have from 0.7 to 1.6 g of firmly adhering soil per root system.

The Gram staining and morphological characteristics of bacterial cultures from true pea rhizosphere, artificial "rhizosphere", and control soils are reported in Table II.

TABLE II

Staining and morphological characteristics of isolates from control, root exudate treated, and rhizosphere soils

Sample	Number of isolates	Gram reaction (%)			Morphology (%)		
		+	—	+/—	Rods	Coccoid rods	Cocci
Pea rhizosphere	95	6.3	88.2	5.5	83.1	10.6	6.3
Control	85	37.6	58.9	3.5	83.3	11.8	5.9
Root exudate treated soil	94	14.5	78.2	7.3	88.3	9.5	2.2
Control	90	30	60	10	90.4	5.3	4.3

These results show that although the change in the balance of organisms is not as great in the root exudate treated soil as in the

true rhizosphere there is a marked increase in the proportion of Gram-negative bacteria. The morphological grouping of the bacteria from the various treatments did not show any differences, in all cases the rod forms predominated. An attempt to subdivide this large group according to length failed due to the pleomorphic nature and variable length of many of the organisms.

(*b*) *Nitrification rates in root exudate treated soils*

(i) Release of ammonia and nitrate from soil organic matter. Ammonia and nitrate estimations were made on soils after treatment with root exudate for three weeks. The results presented in Table III show that for this particular soil the addition of root exudate had no influence on the nitrification rate.

TABLE III

Effect of soil treatment on nitrification

Supplement	NH_3–N (mg N/10 g soil)	NO_3^-–N (mg N/10 g soil)
Without peptone		
Root exudate . . .	0.032 *)	0.61
Nil	0.03	0.61
With peptone		
Root exudate . . .	0.62 **)	1.64
Nil	0.62	1.30

*) Mean of duplicate treatments.

**) Mean of six replicate treatments. S.E. = ± 2.3%.

(ii) Decomposition of peptone in root exudate treated soils. Soils which had been treated for 21 days with root exudate or plant nutrient solution then received peptone solution (20 mg peptone per 10 g soil) and incubated for a further six days. The results (Table III) show the amounts of ammonia and nitrate in the soils at the end of this period.

The higher nitrate content of the root exudate treated soils indicates that these soils support a more active flora than the controls when readily decomposable material, *e.g.* amino acids, is present. The greater accumulation of nitrate but not ammonia in root exudate treated soils shows that the nitrification rate is increased. This may be due either to the direct stimulation of the nitrifying organisms by root exudate or to the greater supply of ammonia from the rapidly decomposing amino acids.

(c) *Phosphate release in root exudate treated soils*

The phosphate content of soils which had received root exudate for fourteen days was compared with those of control soils, and as the results in Table IV show there were no changes due to treatment.

TABLE IV

Phosphate content (ppm P) in treated and untreated soils			
Soil	Treatment	Extractant	
		1% citric acid	2% HCl
A	Root exudate	0 *)	32
	Control	0	32
B	Root exudate	7	164 **)
	Control	8	181

*) Mean of duplicate treatments. **) Not significant.

As the rhizosphere population could play an important role in the release of phosphate from more readily decomposable organic compounds, the phosphate liberation from yeast nucleic acid was investigated (Table V).

TABLE V

Release of phosphate from nucleic acid added to soil (mg P)		
Treatment	Extractant	
	1% citric acid	2% HCl
Root exudate	0.89 *)	1.05
Control	0.90	1.07

*) Mean of four replicate treatments.

In all samples there was a rapid release of phosphate with no differences due to treatment. It is possible that if the experiment had been continued differences may have occurred. This was not done as the study was carried out in a soil suspension which, over a longer period, could produce reactions not due to the initial populations.

(d) *Effect of root exudate on soil respiration*

The oxygen uptake of treated and untreated soils before and after the addition of glucose are summarized in Figures 1A and 1B.

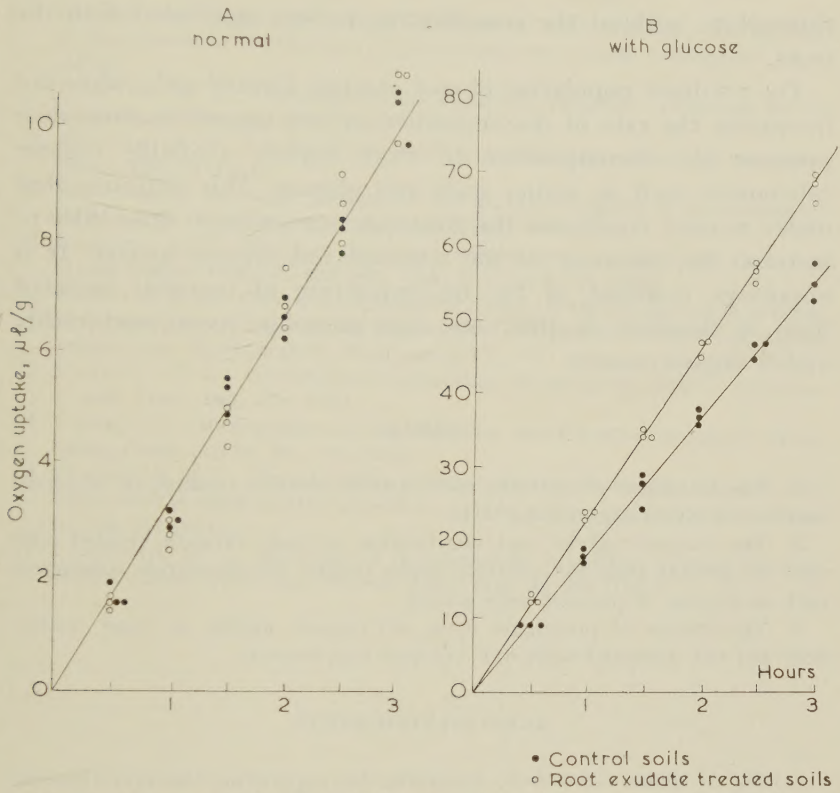

Fig. 1. Oxygen uptake of control and root exudate treated soils at 30°C.

These results make it quite apparent that, although the treatment with root exudate did not influence the normal oxygen uptake of the soil, the decomposition of added glucose was more rapid.

DISCUSSION

The selective stimulation of Gram-negative bacteria in soil by root exudate supplements indicates that the technique is suitable for obtaining soil with the characteristics and metabolism of that surrounding plants roots. Although rhizosphere conditions such as CO_2 accumulation, mineral uptake, and the physical nature of the root surface do not operate in root exudate treated soils, the properties will be indicative of those of the true rhizosphere. By preparing soils in this way it is possible to study conditions in the

rhizosphere without the complicating factors associated with the roots.

The resultant population of root exudate treated soils, while not increasing the rate of decomposition of soil organic matter, does promote the decomposition of more readily available organic substances such as amino acids and glucose. This indicates that under normal conditions the rhizosphere population does little to increase the turnover of the resistant soil organic matter. It is important, however, in the decomposition of material excreted from, or sloughed off, the roots, dead microbial tissue, and freshly added organic matter.

SUMMARY

1. The treatment of soil with root exudate solution resulted in increased numbers of Gram-negative bacteria.

2. The oxygen uptake and nitrification of root exudate treated soils were no greater than the controls unless readily decomposable substances such as glucose or peptone were added.

3. The release of phosphate from soil organic matter or yeast nucleic acid was not increased with root exudate supplements.

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THE EFFECTS OF VARIOUS SOIL TREATMENTS ON THE OCCURRENCE OF MARSH SPOT IN PEAS AND ON MANGANESE UPTAKE AND YIELD OF OATS AND TIMOTHY

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Earlier work (Heintze[5]) had pointed to the importance of the nitrogen status of the soil in connection with the occurrence of manganese deficiency in the field, and had demonstrated that the defect in peas known as marsh spot was associated with the nitrogen and manganese status of soils and plants. The following experiments were designed to study the effects of applications of zinc and molybdenum on manganese availability, and the differences in availability of certain manganese materials varying in solubility and reducibility. In the latter case oats and timothy were used as test plants, while the occurrence of marsh spot in peas was used as an indication of manganese availability in the presence of other cations. The results are therefore grouped in two parts.

PART I

EFFECT OF MANGANESE SULPHATE, SODIUM MOLYBDATE AND ZINC SULPHATE APPLICATION TO SOILS ON THE OCCURRENCE OF MARSH SPOT IN PEAS

This series was based on the supposition that other ions besides manganese and nitrogen may influence the metabolic disturbances leading to marsh spot in peas. Roach and Barclay[20] found the yield of potatoes growing on a manganese-deficient soil to be increased by applications of zinc sulphate, whilst Hewitt[7] observed symptoms in sugar beet resembling those of manganese

deficiency as a result of applying zinc in excess to a manganese-deficient soil.

Results from studies by Mulder[16] and Millikan[15] have shown that molybdenum is associated with nitrogen metabolism in plants and that the molybdenum content of legumes is large compared with cereals. In water culture studies Warington[23] observed no effect on the occurrence of grey speck in oats from addition of molybdenum, and found that the uptake of either manganese or molybdenum was independent of the presence of the other.

The following experiments on various soils included the application of sodium molybdate, zinc sulphate and manganese sulphate.

Experimental

With the exception of a mineral, alkaline soil (Rothamsted), which had a high content of manganese higher oxides, all the soils used were known to be manganese deficient under field conditions, and were found to be low in total manganese and alkaline in reaction. The manganese-deficient soils were 3 fens, Isleham, Burwell, Swaffham; a loam, Tring, and a marsh soil, Ivychurch. In the first series a basal dressing of NPK was given as ammonium nitrate, potassium phosphate and potassium sulphate. 250 ppm Mn as manganese sulphate and either 100 or 50 ppm Mo as sodium molybdate were added in solution to the soils when the plants were beginning to flower. 200 ppm Zn as zinc sulphate was applied a few days before sowing. Using a 1 : 1 mixture of a manganese-deficient fen soil and acid- and water-washed flint, a second series tested the effects of sodium molybdate in the presence of either calcium nitrate or urea. The peas, variety Onward, were usually sown in spring and harvested some three months later, but the series with urea was grown during autumn. Three plants were grown in each pot and treatments were replicated three times. The experiments were carried out during the years 1948, 1949, 1950, 1952.

Manganese analyses of the plants and seeds were carried out after wet digestion with perchloric acid, the manganese being determined as permanganate after oxidation with potassium periodate. Molybdenum was determined as thiocyanate using the method of Dick and Bingley[3].

Results

Observations on signs of manganese deficiency and molybdenum toxicity were made during the growing period. Addition of molybdate to manganese-deficient soils caused bronzing of the leaf edges shortly after application and gradually the leaves became bright yellow all over. The plants reached maturity but the seeds were in some cases small and yellow coloured. Leaves of plants on an untreated manga-

nese-deficient fen soil (Isleham) showed symptoms of manganese deficiency, while healthy plants were maintained on the Rothamsted soil under all treatments. The effects of the various treatments on the occurrence of marsh spot in the peas and on their total contents of manganese and molybdenum are summarised in Table I.

TABLE I

Effect of manganese sulphate, sodium molybdate and zinc sulphate applied to soils on the occurrence of marsh spot in peas and on their total manganese and molybdenum contents

Series A: Application of manganese sulphate, sodium molybdate, and zinc sulphate. Nitrogen added as ammonium nitrate

Series B: Application of sodium molybdate. Nitrogen added as calcium nitrate or urea.

Series A

Treatment	ppm Mn,Zn, or Mo	Isleham 1948		Burwell 1952		Tring 1948		Ivychurch 1948		Rothamsted not Mn-def. 1949	
		Marsh spot intensity									
		No. peas	% M.sp.	No. peas	% M.sp.	No. peas	% M.sp.	No. peas	% M.sp.	No. peas	% M.sp.
None	—	55	0	38	17	72	16	65	3	45	0
Mn	275	64	0	60	0	67	0	—	—	—	—
Mo	100*	43	54	29	25	31	100	30	3	46	0
Zn	200	25	8	—	—	—	—	—	—	33	0
		Mn and Mo content ppm									
		Mn	Mo	Mn	Mo	Mn	Mo	Mn	Mo	Mn	Mo
None	—	9	7	16	—	5	8	8	10	—	—
Mn	275	29	—	51	—	10	8	—	—	—	
Mo	100	5	675	12	—	—	600	5	890	—	—
Zn	200	5	–	—		—		—	—		–

Series B

Treatment	ppm Mo	Mixture of Swaffham + quartz 1950			
		Marsh spot intensity		Mn and Mo content, ppm	
		No. peas	% M.sp.	Mn	Mo
Calcium nitrate	—	58	19	10	10
,, ,, +Mo	50	39	10	10	360
Urea	—	61	39	13	15
,, + Mo	50	50	0	13	465

* 50 ppm, Burwell.

Peas affected with marsh spot were found on all manganese-deficient soils with the exception of a fen soil (Isleham), and here the foliage showed signs of manganese deficiency. This latter result is contrary to earlier findings, both in pots and in the field, since manganese deficiency had invariably been evidenced only in the peas themselves. Since the metabolic processes resulting in necrotic pea seeds are as yet not fully understood, these results may indicate that different reactions within the plant are responsible for preventing manganese deficiency symptoms in leaves and in seeds. Alternatively the critical time for manganese uptake may differ. It has previously been shown (H e i n t z e [5]) that to secure healthy peas manganese must be available from the flowering stage onward, and that if the supply is cut at flowering time the prior accumulation of manganese does not prevent marsh spot.

Addition of manganese sulpate secured healthy peas on all soils. The effect of sodium molybdate was not consistent. While healthy peas were obtained on an alkaline, manganiferous mineral soil (Rothamsted) when molybdate had been added considerable increases in the incidence of marsh pot were found on some manganese deficient soils. Thus on Isleham fen, where in spite of chlorotic plants healthy peas were harvested from the control, about 50% of the peas were affected where the soils had received molybdate. On a mixture of quartz and Swaffham fen, where the source of nitrogen was either urea or calcium nitrate and where the peas were affected with marsh spot, molybdate application decreased the incidence particularly in those pots treated with urea. Application of zinc sulphate to a fen (Isleham) carrying healty peas resulted in affected peas with unusually large necrotic areas, but its addition to a mineral soil (Rothamsted) well supplied with manganese was without effect. The result on the deficient Isleham soil agrees with findings in experiments with sugar beet by H e w i t t [7].

The total manganese content of ripe peas was generally decreased by molybdate application, except in the series where the peas were grown in late autumn on a soil-quartz mixture with either calcium nitrate or urea; here the manganese content remained the same. The molybdate content of the pea seed was increased to a greater extent by addition of molybdate to the soil than was the manganese content by larger dressings of manganese.

The total manganese content of peas affected with marsh spot

varied from soil to soil and differences in this respect between necrotic and healthy peas were sometimes small, a result agreeing with previous findings [5]. The haulms from plants on Isleham were analysed; their manganese content remained unaltered by molybdate applications. On this soil added manganese increased the manganese content of seed only from 9 to 29 ppm, but the corresponding increase in haulms was from 11 to 82 ppm, a finding similar to earlier results. Qualitative comparison between the amino acid contents of healthy and affected peas by chromatography showed that the content was greater in the diseased peas. Molybdate application to the soils increased the amount of amino acid in peas, which agrees with the results of Hewitt *et al.* [10] obtained with other crops. The amounts were greater in peas grown on organic soils than on mineral soils. Glutamic acid was found to be predominant in all analyses, but the identified compounds included also aspartic acid, glycine, threonine, alanine and asparagine.

It has been shown that manganese within the plant, besides being associated with nitrate assimilation (Jones *et al.* [13], Burström [2]) also plays a part in plant respiration (Lundegårdh [12]). In collaboration with Dr. P. J. G. Mann of the Biochemistry Department some preliminary determinations were made of the rates of oxygen uptake by air-dried pea seeds grown on two manganese-deficient soils which had been variously treated. Oxygen uptake was measured manometrically with the Warburg apparatus. The seeds had been stored in air-dry condition over a considerable period, but the results from the two soils were consistent. Powdered peas were suspended in *M*/15 neutral phosphate solution and the measurements made over a period of one hour. The results can be summarised briefly. The greatest oxygen uptake was found with peas which had been grown on molybdate-treated soils. The uptake of peas from untreated soil was 14–17% less, and that of peas from manganese treated soils 34–39% less than that of corresponding peas from soils treated with molybdate.

Small additions of manganese (16.5 μg Mn^{++} to 0.2 g. of material) to the pea suspensions had little or no effect on the oxygen uptake of those peas from soils treated with either manganese or molybdate, but increased considerably the uptake of peas from untreated soils, particularly in the first ten minutes, when increases of 30–40% were measured. The results show that in the peas affected with marsh spot,

and derived from untreated soil, substances had accumulated which were readily oxidised in the presence of manganese. Such substances were not present in peas from soils with added manganese sulphate or sodium molybdate.

In a later experiment freshly gathered peas were used which were only slightly affected with marsh spot; those grown on untreated soil contained considerably more manganese than the corresponding peas from the experiments just described. In this case no differences were found in the oxygen uptakes of peas from untreated and from manganese- or molybdenum-treated soils.

Soil analyses

Since application of molybdate or zinc sulphate to the soils showed no effects on either the ammonium acetate, the neutral or the alkaline pyrophosphate soluble manganese, estimated at the end of the experiment, the soil analyses are not tabulated. No estimated fraction could be related to the presence or absence of marsh spot in the peas.

Discussion

The above results, although of a preliminary nature, show that additions of sodium molybdate and zinc sulphate to manganese-deficient soils generally increase the incidence of marsh spot in peas, while such applications are without effect on a soil with ample supply of manganese higher oxides. The possible association between forms of nitrogen and effect of molybdate is indicated by the decrease of marsh spot incidence by molybdenum addition in the presence of urea, while in the presence of ammonium nitrate on similar soils increases in the occurrence of marsh spot were found. In his study on the effects of excess manganese or molybdenum on flax Millikan[15] has shown that such effects were related to the nitrate and urea but not to the ammonium-nutrition of the plant. Mulder[16] also has investigated the dependence of the molybdenum requirement of plants of the form of the nitrogen supply. The above contrasting results from molybdate application to soils with either ammonium nitrate or urea, would seem to indicate that the molybdate was affecting processes within the plant as well as possibly suppressing manganese uptake; the latter effect is illustrated in one case by a halving of the manganese content of the pea seed.

The evidence accumulated in studies on the essential function of molybdenum in plant life indicates that molybdenum acts as a catalyst in the reduction of nitrate to ammonia, and through this reduction influences the amino acid content of plants. If some of the storage compounds found in pea seeds through this action of molybdenum were readily oxidisable, this would explain why pea suspensions from molybdate treated soils gave higher oxygen uptakes than those from either untreated or manganese-treated soils. Aqueous or pyrophosphate pea suspensions from molybdate-treated soils reduced manganese dioxide considerably more than did pea suspensions from untreated soils. This result is in general agreement with Mulder's [17] finding that the reduction of selenite to metal selenium proceeded more readily in macerated spinach plants adequately supplied with molybdenum than in those which were Mo-deficient. Manganese dioxide was further reduced more effectively by water or alkaline pyrophosphate suspensions of peas from soils given nitrogen in the form of urea than in the form of nitrate. The importance of the nitrogen metabolism in connection with the presence or absence of marsh spot was further shown by a greater accumulation of amino acids in affected peas than in healthy ones. Hewitt *et al.* [10] found that the effects on amino acid content arising from changes in the molybdenum level were most pronounced under manganese-deficient conditions. This agrees with the present result that molybdate application to manganese-deficient soils increased the amino acid content of peas. The effect of applying zinc to a manganese-deficient soil is in harmony with observations by Hewitt [7] that excess zinc produced symptoms in sugar beet similar to those of manganese deficiency. The decrease in total manganese content of peas resulting from zinc application might suggest suppression of manganese uptake, as in the case of the citrus seedlings on acid soils described by Smith and Specht [21]. Soil analyses failed to show any effects of the molybdate and zinc treatments on various manganese fractions, or to relate any one of the fractions to the presence or absence of marsh spot. The varying total manganese contents of peas affected with marsh spot agree with earlier work [5]. Although low total manganese content of the peas could account for the appearance of the disorder on some soils, previous and present results seem to suggest that the development of necrosis may be determined by the relation of manganese content, or some fraction thereof, to

certain products of nitrogen metabolism rather than by the manganese content alone.

PART II

It has long been known that soluble manganese salts applied to alkaline organic soil quickly become unavailable to plants. In the following series of investigations attempts were made to prolong the availability of manganese salts by applying them as crystals or as crystals protected by a coating designed to delay their conversion to less available forms. The possibility was also tested of using manganese higher oxides which would become available by reduction. One series included further soil treatments which, although not adding manganese, were of a type which has been shown on some soils to enhance the availability of existing manganese. Two test plants were used. In 1950 oats were chosen as a crop sensitive to manganese deficiency, reacting to it by developing grey speck; by its use the effects of soil treatments might be estimated in the yields of grain; in 1952 a grass crop, timothy, enabled the effects of soil treatments on manganese uptake and on yields to be followed in successive cuttings over a long growing period.

EFFECT OF APPLYING VARIOUS MANGANESE MATERIALS AND OF STEAM OR FORMALIN TREATMENT ON THE YIELD AND MANGANESE CONTENT OF OATS

Experimental

The soil used was a peaty skirt fen soil, known to be manganese deficient, with pH value 7.2 and with a total manganese content of 230 ppm. The soil was mixed with a small amount of silver sand. The sand had been acid washed and subsequently washed free from acid with water. A basal dressing of NPK was given before sowing, supplying 50 mg N as ammonium nitrate, 50 mg K and 75 mg P as potassium sulphate and dihydrogen potassium phopshate. Where potassium permanganate was added allowance for added K in this form was made. Manganese materials tested at two levels, (50 and 100 mg Mn per pot containing about 500 g soil-sand mixture) included manganese sulphate in three forms, namely in solution, as crystals (1–3 mm) and as crystals previously coated with amino plastics, manganese dioxide as ground mineral pyrolusite and potassium permanganate both in solution and as crystals (1–3 mm). Manganese sulphate as solution was tested at an additional level of 25 mg Mn per pot. The materials were applied a few days before sowing, either incorporated throughout the pot (called uniform hereafter) or mixed only into

thet op layer, 1–1½ inches deep (placed). Two non-manganese supplying treatments were further tested: *1*) steam sterilization of the soil at 100° for about half an hour and *2*) treatment of the soil for a few days with dilute formalin solution. Soil treatments were replicated four times, and two replicates used to each of the placement technique. Oats were sown at the end of April, at the rate of six seeds per pot; after three weeks growth they were thinned to three plants, which were harvested at the end of July. The variety used was Onward, known to be sensitive to manganese deficiency.

Deficiency signs of the plants

Grey speck was first observed on plants some 3 weeks old growing on the untreated, formalin and manganese dioxide treated soils, but after this time manganese deficiency signs developed rapidly in all treatments. Observations made from the end of June to the close of the experiment showed that no treatment was successful in controlling manganese deficiency signs in the leaves of the main stems or in tiller leaves. Plants of all treatments exhibited grey speck of varying severity.

At the end of June leaves of tillers but not of the main stems exhibited signs of copper deficiency in soils which had either received manganese materials or been steam sterilized. Plants from control or formalin-treated soils remained free from signs of copper deficiency throughout the experiment. The occurrence of copper deficiency, where manganese had been added or the soil had been treated with steam, with subsequent liberation of readily soluble manganese, points to an interaction between manganese and copper similar to that observed by Ödelien [19] on copper-deficient soils. So far little is known of the effect of manganese additions on copper availability of the soil. The fact that only those leaves growing on tillers exhibited signs of copper deficiency might indicate that the availability or the uptake of copper was affected only at a later stage.

Effects of soil treatments on yields of grains, leaves, husks + stems and the manganese content of grains and leaves

At harvest the grains, leaves and husks + stems were dried and analysed separately.

Dry matter yields of the plants removed in the early thinning, were less than 0.5 g and showed no outstanding differences between treatments. The effects of treatments on final yields of grains, leaves and husks + stems are summarised in Table II, where increases over

TABLE II

Increases in weights of grains, leaves and husks + stems over controls from addition of various manganese materials to the soil and from formalin and steam treatments

Increases in g dry matter over control

Treatment	Grain	S.E. Diff.	Leaves	S.E. Diff.	Husks + Stems	S.E Diff.
Control	3.77 (S.E. ± 0.241)		1.36 (S.E. ± 0.085)		3.50 (S.E. ± 0.117)	
$MnSO_4$ solution	0.69*	±0.318	0.16	±0.110	—0.16	±0.151
$MnSO_4$ crystals	0.57	±0.341	0.33*	±0.120	—0.83**	±0.166
$MnSO_4$ cemented crystals	1.24**	±0.341	0.43**	±0.120	—0.75**	±0.166
Pyrolusite	1.12**	±0.341	0.59**	±0.120	—0.92**	±0.166
$KMnO_4$ solution	1.46**	±0.341	0.70**	±0.120	—1.02**	±0.166
$KMnO_4$ crystals	0.84*	±0.341	0.64**	±0.120	—0.98**	±0.166
Formalin	0.31	±0.405	0.35*	±0.147	—0.87**	±0.203
Steam	2.50**	±0.405	1.14**	±0.147	—0.81**	±0.203

the controls, ignoring placing and bulking rates, are set out; while in Fig. 1 total yields of various parts of the oat plant above ground from all manganese sulphate treatments are compared with total yields from other manganese forms, at low or high manganese level and with materials either placed or uniformly distributed. Despite the failure of any treatment wholly to control manganese deficiency signs on the plants it will be seen from Table II that with two exceptions all treatments increased the yields of grain significantly. The outstanding treatment was steam. Although the beneficial effects from steam treatment on the grain yield may be explained partly by increases in readily soluble manganese and by a temporary removal of micro-organisms competing for available manganese it is clear from the magnitude of the increase that other factors beside manganese availability may have been of greater importance. It seems possible that steam treatment may have raised the general fertility level of the soil. It is well known that this treatment increases the available nitrogen of the soil considerably, and Aldrich and Martin[1] found increases in readily soluble calcium, magnesium and potassium besides manganese. The small magnitude of the increase in grain yield from formalin treatment is contrary to results by Steenbjerg[22]. The highly significant increase from pyrolusite indicates the importance of manganese higher oxides as possible sources of available manganese on this type of soil. The increases in yields of leaves were similar to those of grain, except for the fact that formalin treatment effected a highly significant increase here. All treatments depressed the yields of husks and stems. If the effects from the various manganese materials are grouped as effects from manganese sulphate, whether applied as solution, crystals or coated crystals, and as effects from "other manganous forms" (comprising pyrolusite and solution or crystals of permanganate) certain contrasts are evident. Thus "other manganese forms" show a greater effect on the weight of leaves and on the total yield of the above ground parts. Effects on husks + stems go in the opposite direction to those on grain, all treatments reducing this fraction. The "other forms" of manganese are again more effective than manganese sulphate in reducing the amounts of husks + stems. Taking these two features together, as for instance by using the ratio of husks + stems to leaves, the difference between the behaviour of the two groups of manganese materials becomes very highly significant. A difference

in the same sense as for leaves (again between the two groups of materials) is found in the case of the grain, although it is below the level of significance in this instance. From Fig. 1 it can be seen that the two rates of application on the whole give very similar results, suggesting that within the limiting conditions of the experiment the lower rate was almost sufficient for maximum effect. This would

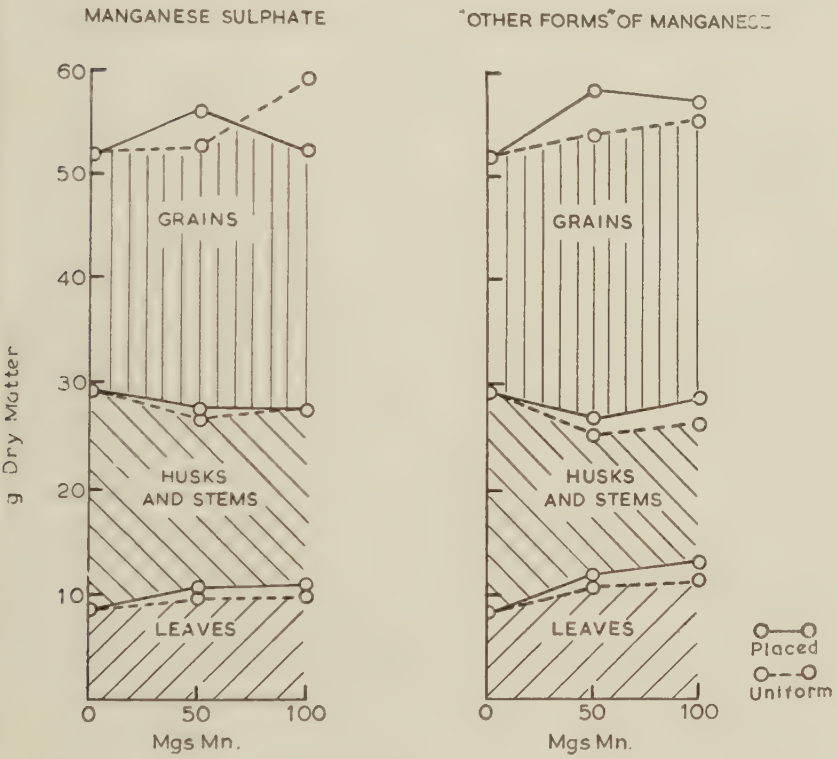


Fig. 1. Total yields of various parts of oat plants grown on soils receiving manganese sulphate or other forms of manganese at low and high manganese levels and either placed or uniformly distributed through the soil.

seem anomalous when it is considered that in no case were plants obtained wholly free from signs of manganese deficiency. If these observations should be substantiated and the maximum effect can indeed be reached without removing the deficiency signs then the use of such symptoms for prescribing treatment is considerably weakened, and would need reinforcement from additional knowledge

The only significant exception to this seeming equivalence of the two rates was the unexpected fall in grain yield at the higher level when manganese was added in the form of manganese sulphate, and placed only in the top layer of soil, as can be seen from the comparison in Table III where the relevant data are set out.

TABLE III

Mean yields of grain at two rates of manganese sulphate either placed or distributed throughout
g per pot

Treatment	Placed	Uniform
Control	3.77	
$MnSO_4$ mean 50 mg Mn	4.82	4.15
$MnSO_4$ mean 100 mg Mn	4.29	5.19
Interaction $+1.57$ ($P < 0.01$)		

No satisfactory explanation can be presented of the highly significant differences in behaviour between placed and distributed manganese sulphate at two levels, but the results seem to suggest that the largest dose of placed manganese sulphate has exceeded the bounds of greatest usefulness. The average dry matter weight per grain of the control pots was 0.0264 g with a range of 0.0243–0.0283 among treatment means, suggesting that the grain size throughout the experiment conformed well to a norm. Analysis of grain and leaves showed that the manganese contents were significantly correlated with yields, the coefficients being $+0.590$ for grain and $+0.587$ for leaves on 15 pairs ($P < 0.02$). The values are not high for causal relationship but they suffice to demonstrate that the manganese contents were at a level, where the amounts influenced development.

Soil analyses

Soil analyses shortly after steam treatment of the soil showed some increases in both neutral ammonium acetate and alkaline pyrophosphate soluble manganese. Thus 18 ppm ammonium acetate soluble and 75 ppm alkaline pyrophosphate soluble manganese were found as compared with 2 and 40 ppm respectively for the untreated soil. Analyses of soils at the end of the experiments showed that differences in amounts of manganese soluble in the above extractants from untreated and from steam-treated soils were considerably less.

No treatment had increased the content of readily soluble manganese and only steam-treated soil had a lower pH value, 6.8 compared with pH 7.2 of the untreated soil. Of manganese added to the soil as pyrolusite or as permanganate 45% and 30% respectively were recovered as soluble in ammonium acetate with 0.2% hydroquinone. Corresponding recovery values were 4% and 30% in extracts with alkaline pyrophosphate. No manganese fraction reflected differences in yield increases from the application of various manganese materials.

AVAILABLITY OF VARIOUS MANGANESE MATERIALS TO TIMOTHY

The results from the experiment with oats indicated the possible usefulness of sparingly soluble manganese materials as a source of potential available manganese. Some results of Jones and Leeper[11] and Jones[12] further supported this view. Since the rate of release of available manganese from such materials may be of importance the test plant in the experiment to be reported was timothy grass, which by providing several cuts allowed the manganese assimilation to be followed over a considerable period.

Experimental

The soil, an alkaline sandy loam with shell fragments and low in total manganese, had in previous years carried crops showing signs of severe manganese deficiency. Timothy (*Phleum pratense*) of the Welsh Plant Breeding strain S. 48 was sown at the end of April in the soil-quartz mixture in glass pots of about ½ kg capacity. The watering was done from below by standing the pots in saucers filled with water, a technique which successfully prevented rotting of the sod. At times it may have tended to create reducing conditions in the soil. The following manganese materials were tested, being mixed throughout the soil before sowing; manganese sulphate crystals (2–3 mm), potassium permanganate crystals (2–3 mm), ground pyrolusite, synthetic hausmannite, γmanganite and γmanganese dioxide. Hausmannite and γmanganite were synthesized according to Feitknecht and Marti[4]. X-ray analyses showed these products to possess structures corresponding to those of naturally occurring hausmannite and manganite, while the reflexions from a manganous manganite, a very imperfectly crystalline preparation formed by the slow addition of manganese sulphate solution to a solution of calcium permanganate, were in harmony with those from γ MnO_2. The n values of MnO_n in these three materials were respectively 1.35, 1.48 and 2.06. Manganese sulphate in solution was added outside the soil to the saucer either at the time of sowing or after the first or second cut. The

manganese was added at two levels, namely 100 and 200 mg. A basal dressing of NPK was applied as ammonium nitrate, potassium sulphate and calcium phosphate. Nitrogen applications were repeated after the second and fourth cuts. Three replicates were used, and the timothy was sown at the end of April. At intervals of about 2–3 weeks, the grass was cut down to within 3 inches from the soil, the first cut being taken at the beginning of June.

Results

No signs of manganese deficiency as described by Williams [24] were noticed. The effects of the various treatments on yield were small and did not reach the level of significance. The amounts of manganese recovered in the cuts are set out in Table IV, the totals from the first three and from the three subsequent cuts being shown for the two levels of supply.

TABLE IV

Manganese recovery from timothy in six cuts
mg manganese per pot

Treatment	1–3 cuts		4–6 cuts		1–6 cuts	
	100 mg Mn/pot	200 mg Mn/pot	100 mg Mn/pot	200 mg Mn/pot	100 mg Mn/pot	200 mg Mn/pot
$MnSO_4$ solution at sowing	1.10	2.57	1.32	2.98	2.42	5.55
$MnSO_4$ crystals	1.47	2.85	1.74	2.08	3.21	4.93
γMnO_2 (synth.)	0.72	1.08	1.46	2.44	2.18	3.52
MnO_2 pyrolusite	0.71	0.55	1.11	1.43	1.82	1.98
Hausmannite (synth.)	—	1.26	1.25	2.10	—	3.36
γManganite (synth.)	0.59	0.57	1.08	1.94	1.67	2.51
$KMnO_4$ crystals	0.42	0.47	1.53	1.40	1.95	1.87
None	0.47		0.79		1.26	

The highest manganese uptake on this soil was from manganese sulphate added either in solution or as crystals. In the case of the crystals more manganese was recovered from the higher supply level in the earlier cuts than in the later, while the reverse occurred for all other materials applied before sowing. From manganese sulphate solutions applied after the first or second cut the highest recovery was secured in the cut immediately following application, the content decreasing in the later successive cuts, as in the case of manganese sulphate crystals at the high supply level. Recoveries from permanganate were similar to those from pyrolusite.

Jones and Leeper [11] and Jones [12] showed that the reducibility of manganese higher oxides in aqueous quinol was

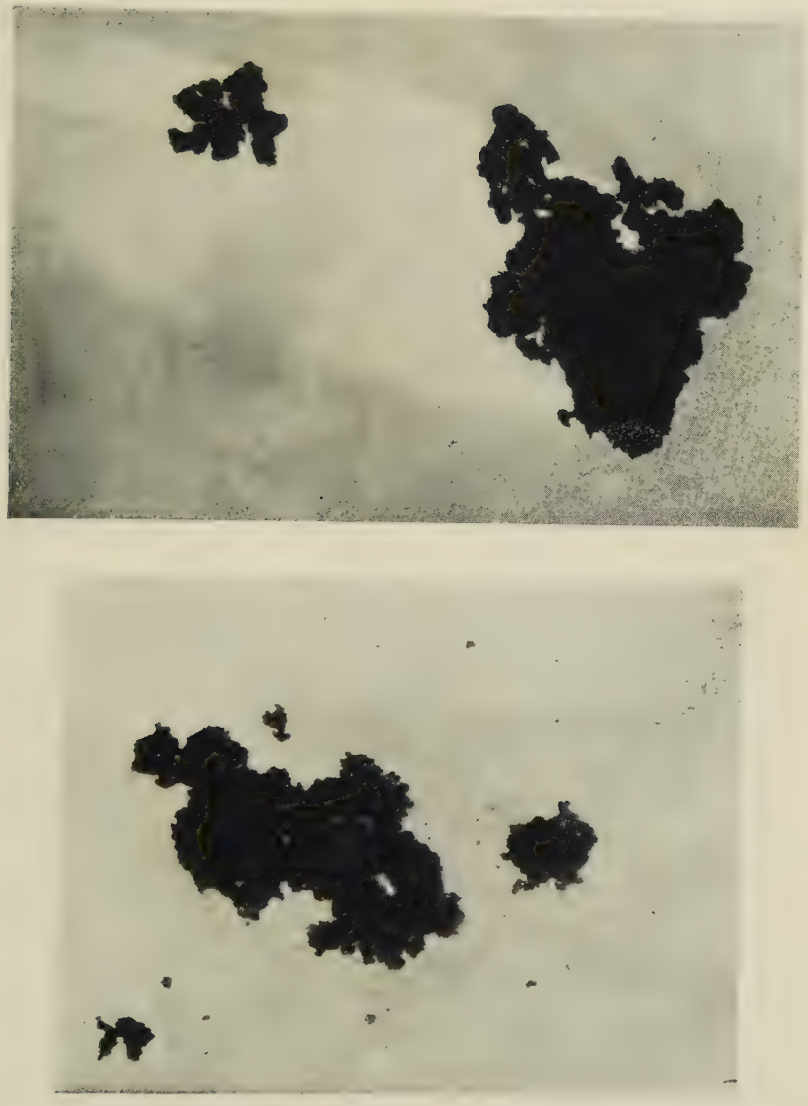
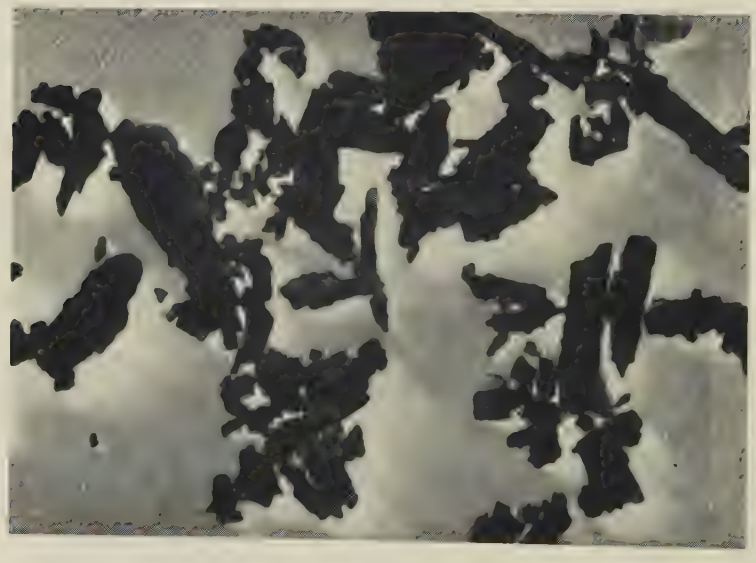


Plate I. Electron micrographs of manganese higher oxides (× 18000). A: γ Manganese dioxide. B: Hausmannite.

A



B

Plate II. Electron micrographs of manganese higher oxides (× 18000). A: Manganite. B: Pyrolusite.

correlated with their ability to supply manganese to oats and peas. The order of decreasing reducibility of the manganese higher oxides used in this experiment was as follows: synthetic γmanganese dioxide, synthetic hausmannite, synthetic γmanganite, mineral pyrolusite. From Table IV it is evident that the order of decreasing manganese uptake by timothy was the same. The behaviour of the synthetic hausmannite in this experiment was contrary to that found by Jones and Leeper[11], for it was readily reducible and also provided available manganese. In their work Jones and Leeper[11] emphasized the importance of various physical properties of the higher oxides in connection with their manganese availability to plants. The sizes of the ultimate particles of the three synthetic manganese higher oxides together with that of the mineral pyrolusite are shown in the electron micrographs of Plates I and II. It will be seen that there is a general agreement between the availability as measured by manganese uptake by timothy and the specific surfaces. Thus the contrast between synthetic γmanganese dioxide and pyrolusite is confirmed by the electron micrographs. The micrograph of the well crystalised hausmannite preparation resembles that of the very poorly crystalline γmanganese dioxide. The higher manganese uptake from manganite than from pyrolusite, which yielded similar amounts of manganese in aqueous quinol, might be connected with the higher specific surface of the former material.

Despite the failure to obtain any effects from the manganese materials on the yield of timothy, the increases in manganese uptake from the higher oxides confirm Jones and Leeper's[11] claim, that such oxides are able to consitute available sources of manganese. The degree of availability was related to their reducibility and to physical properties such as specific surfaces.

Discussion

The above results show that manganese materials other than the commonly used sulphate may prove of importance as available sources of manganese to plants on soils which convert soluble manganese salts into unavailable forms. Although the higher supply level of placed manganese sulphate decreased the grain yield the superiority of placed over uniformly distributed manganese materials is evident from the dry matter yields. This result may become of

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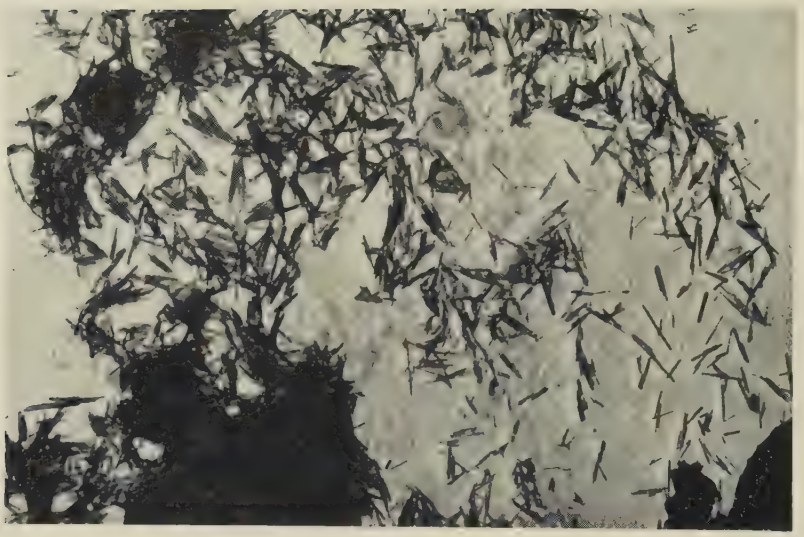

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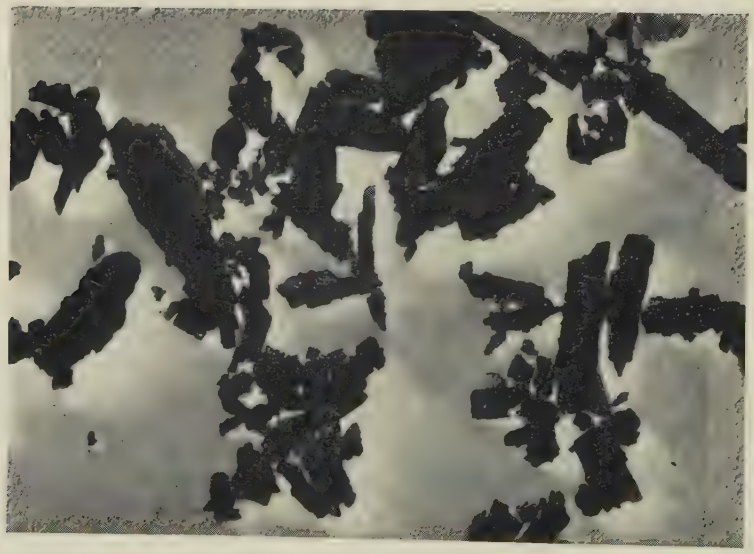

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practical value if confirmed under field conditions. The inability of manganese materials to prevent deficiency signs in the oat plant at the same time as they effectively increase the yield, while in the pea plant often no deficiency signs are visible on the leaves of plants whose seeds are heavily affected with marsh spot, raises the problem whether the same manganese fraction that governs the maintenance of healthy leaves also effects increases in grain yield of oats or determines the presence or absence of marsh spot in peas. The results from the oat experiment also show that signs of manganese deficiency in this crop are not necessarily a reliable guide to the need for further manganese application. In a field experiment on a manganese deficient soil in which oat plants were sprayed with manganese sulphate solution, Nicholas and Fisher[18] found that such treatment increased the final yields of grain significantly but that the plants exhibited grey speck late in the season after spraying had been discontinued. The occurrence of copper deficiency apparently being induced in oat plants by manganese application to a manganese-deficient soil not hitherto known to be copper deficient, taken together with the effects on peas of adding zinc or molybdate to manganese-deficient soils, demonstrates the importance of interactions between various metals in the study of deficiencies. In earlier work Heintze and Mann[6] suggested the possible importance of interaction between various trace elements based on the ability of various metals to replace manganese in the soil, and Heintze[5] furnished some evidence suggesting that manganese deficiency was associated with the nitrogen status of the soil and of the pea plant. In that case it was shown that raising the nitrogen content of the plant by injection with simple nitrogen compounds produced marsh spot while non-treated plants on the same soil carried healthy peas.

Other workers, *e.g.* Hewitt[7 8 9] and Smith and Specht[21] have also shown the considerable influence by one metal on the action of another in connection with studies on manganese deficiency and iron chlorosis. The results obtained here from the use of manganese higher oxides agree in general with those described by Jones and Leeper[11] and Jones[12]. The availabilities of these oxides were related to their chemical activities and specific surfaces. Increases in dry matter yields or in manganese assimilations could not be correlated with results from soil analyses in which various manganese fractions were estimated.

SUMMARY

1. Application of sodium molybdate or zinc sulphate to manganese deficient soils increased the incidence of marsh spot in peas but was without effect on peas grown on a mineral manganiferous soil.

2. On some soils the total manganese content of pea seeds was reduced by molybdate or zinc application.

3. Peas affected with marsh spot contained higher amounts of amino acids than healthy peas and addition of molybdate to the soil increased the amino acid content of peas.

4. Manganese dioxide was reduced more readily by suspensions of peas from soils with added molybdate or urea than from untreated soils.

5. Significant increases in grain and leaf yields of oat plants were obtained on a manganese-deficient soil by application of various manganese materials which failed to prevent the occurrence of grey speck.

6. Steam treatment of the soil proved more effective in increasing dry matter yield of grain than any manganese treatment.

7. Placement of manganese materials was on the whole more effective than when the materials were incorporated throughout the soil.

8. Steam treatment or manganese application induced copper deficiency in oat plants grown on a manganese-deficient soil not previously known to be copper deficient.

9. Applications of synthetic manganese higher oxides and the mineral pyrolusite to a manganese deficient loam failed to increase dry matter yields of successive cuts of timothy. The manganese recovery however was related to reducibility and to the size of the ultimate particles of the oxides.

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ON THE EFFECT OF FERROUS SULPHATE ON THE AVAILABLE MANGANESE IN THE SOIL AND THE UPTAKE OF MANGANESE BY THE PLANT II

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INTRODUCTION

The results of pot culture experiments with barley and sugar beet carried out in 1950 and 1951 supported the supposition that the application of ferrous sulphate to certain soils may have the same effect on dry matter yield as addition of manganese sulphate. It was found desirable to extend these investigations because the experiments had been restricted to the application, at one level only, of manganese- and iron sulphates to two loams and one muck soil, using two test crops. The investigations reported here include pot culture experiments carried out in 1952 with oats grown on two sandy soils, containing different amounts of organic matter, and one moor soil. To one of these soils increasing amounts of manganese and iron as sulphates were added and the residual effect of application of iron estimated.

In experiments where dry matter yields and nutrient contents of the dry matter — in the present work the iron and manganese content — are determined for increasing levels of supply, a more accurate interpretation of the effect of fertilizer application can be obtained by modifying the method suggested by Steenbjerg [10 11 12 13] for analysing the yield curves.

If ferrous- and manganese-sulphates have identical effects on dry matter yields and total manganese contents of dry matter, the curves representing the relationship between dry matter yields and manganese uptake after application of manganese sulphate and

ferrous sulphate respectively should coincide over the whole range. The results of the present investigation furnish evidence supporting this supposition.

METHODS

The *pot experiments* were carried out in cylindrical zinc pots coated on the inside with paraffin wax. The pots were fitted with external pipes for watering from the bottom of the container. Broken flints were used for drainage material. Two sizes of pots were used; one size held 20 l and had a surface area of about 500 sq.cm; the other size held 10 l and had a surface area of about 300 sq.cm.

The *manganese content of crops* was determined colorimetrically as permanganate after wet ashing with a mixture of sulphuric- and perchloric acids. Divalent manganese was oxidised in the neutralized solution by potassium persulphate in the presence of sulphuric acid and silver ions.

The *content of iron* in the crops was determined according to the method of Szalkowski and Frediani[14].

All *pH measurements* were made with the glass electrode.

The *calcium carbonate* and *carbon* contents of the soils were determined respectively by the use of the Scheibler and Ter Meulen apparatus.

The *total manganese* content of the soil was determined by boiling 50 g of air-dried soil with 200 ml of 20 per cent hydrochloric acid for one hour using a reflux condenser. The mixture was allowed to cool. After addition of nitric acid the extract was evaporated on a water bath and the content of manganese then determined colorimetrically in the same way as in the crop analyses.

The *exchangeable* and *reducible manganese* of the soils were estimated by shaking, for one hour, 50 g of soil with 200 ml *M* magnesium nitrate alone and with 200 ml *M* $Mg(NO_3)_2$ containing 126 mg $FeSO_4.7H_2O$ or 25 mg hydroquinone. Manganese was determined colorimetrically in the filtrates.

The amount of exchangeable manganese extracted by shaking 50 g soil with 200 ml *M* $Mg(NO_3)_2$ agreed with results obtained by the method of analysis suggested by Steenbjerg[8]. In samples from 23 soils, having pH-values ranging from 4.7–8.2 and exchangeable manganese contents ranging from 0.1–35.7 ppm, a comparison of the two methods showed a correlation coefficient of 0.963. The average values for the 23 soil samples, obtained by shaking the soils for one hour with *M* $Mg(NO_3)_2$, were about 30 per cent lower than those found by Steenbjerg's successive leaching method.

EXPERIMENTAL RESULTS

a. Pot experiments 1952

In 1952 pot experiments were carried out with three soils, representing two different soil types, taken from fields near Ringsted, Sjælland, where symptoms of manganese deficiency had been observed frequently in the preceding years. The soils were analysed before the experiments commenced. Some characteristics of the three soils R_1, R_2, R_3 are set out in Table I.

TABLE I

Characteristics of the soils used in the pot experiments						
Soil No.	Soil type	pH	Calcium carbonate %	Carbon %	Total manganese ppm	Exchangeable manganese ppm
R_1	Sand	8.1	2.78	4.45	160	0.7
R_2	do.	8.0	7.56	8.51	280	0.2
R_3	Moor	7.1	0	18.52	225	0.6

The designs of the experiments are set out in Table II for soil R_1 and in Table III for soils R_2 and R_3.

TABLE II

Design of experiment Soil R_1 1952		
Treatment	mg added per pot	
	Mn	Fe
1	0	0
f_1	0	500
f_2	0	1000
f_3	0	2000
m_1	250	0
m_1f_1	250	500
m_1f_2	250	1000
m_1f_3	250	2000
m_2	500	0
m_2f_1	500	500
m_2f_2	500	1000
m_2f_3	500	2000
m_3	1000	0
m_3f_1	1000	500
m_3f_2	1000	1000
m_3f_3	1000	2000

TABLE III

Design of experiment Soils R_2 and R_3 1952		
Treatment	mg added per pot	
	Mn	Fe
1	0	0
f_2	0	500
m_2	250	0
f_2m_2	250	500

The experiment with soil R_1 was carried out in 20-litre pots with five replicates, while for soils R_2 and R_3 10-litre pots were used and the treatments tested in duplicate. "Analar" grades of $MnSO_4.4H_2O$ and $FeSO_4.7H_2O$ were used and applied as solutions. All pots were sown with Sejr oats and watered to a moisture content equivalent to 40 per cent of the total water holding capacity calculated on an air-dry basis.

The soil R_1 received a basal dressing of 7.5 g $NaNO_3$, 2.0 g KH_2PO_4, 2.5 g K_2HPO_4 and 0.5 g $CuSO_4.5H_2O$ added in solution. Half of this quantity was added to the soils R_2 and R_3.

The seeds were sown in the two groups of pots on 17/4 and 18/4 respectively. Between 23/5 and 26/5 the plants were thinned to 25 plants per pot in the experiment on soil R_1 and 16 plants in the experiments on soils R_2 and R_3. The time of ear formation was very uneven. In the case of soil R_1 ears were formed between 30/6 and 16/7, with soil R_2 from 2/7 to 8/7, and with soil R_3 from 1/7 to 6/7. Ear formation began and finished first on manganese-treated soils.

The crops from different treatments ripened at very different times. It was evident that the crops grown on soils at the highest manganese level ripened earliest and most uniformly. The crop on soil R_1 was harvested in the period 18/8–23/8, on soil R_2 from 15/8–23/8 and on soil R_3 on 11/8.

The first symptoms of manganese deficiency were observed in oats on soil R_1 on 23/5. An assessment made on 9/6 revealed symptoms of manganese deficiency in all plants except those at the highest manganese level (m_3) where symptoms appeared in more than half the plants. The occurrence and intensity of the symptoms suggested that the soil R_1 was acutely deficient in manganese.

On soil R_2 the first symptoms of manganese deficiency appeared during the last days of May. On 12/6 all plants from treatments 1 and f_2 showed symptoms; the growth in treatments m_2 and m_2f_2

was much better although some leaves showed symptoms of manganese deficiency. Soil R_2 would therefore appear to be less deficient in manganese than soil R_1. A few symptoms of manganese deficiency were observed during the first week of June in the oats growing on soil R_3. Apparently manganese deficiency did not affect growth on this soil as all plants grew vigorously in all pots.

Symptoms of iron deficiency were not observed in any of the three experiments during the growing season.

On 3/7 growth of the plants in the three series was assessed according to an arbitrary scale, 1 being the poorest growth and 5 representing vigorous crops. Table IV shows the average scores for each treatment.

TABLE IV

Assessment of growth of oats on July 3rd, 1952			
Treatment	Mean scores *)		
	R_1	R_2	R_3
1	1.0	1.0	5.0
f_1	1.0		
f_2	1.2	1.0	5.0
f_3	1.8		
m_1	2.0		
m_1f_1	3.0		
m_1f_2	2.6		
m_1f_3	3.0		
m_2	2.6	4.5	5.0
m_2f_1	3.2		
m_2f_2	3.0	5.0	5.0
m_2f_3	3.6		
m_3	4.0		
m_3f_1	4.6		
m_3f_2	4.6		
m_3f_3	4.8		

*) Scoring in arbitrary units: 1 = poor growth, 5 = best growth.

These results are in agreement with the results of the assessment of plants with manganese deficiency symptoms made on 12/6 and reveal acute manganese deficiency in soil R_1 where stunted growth was obtained even where 1 g manganese was applied to 20 kg soil. In soil R_2 normal growth was obtained following application of moderate amounts of manganese. The growth in soil R_3 was apparently not checked by the slight deficiency of manganese reflected by a few symptoms.

Plates I, II and III are photographs of the crops, taken on 9/7, to illustrate the gradation of manganese deficiency of the three soils.

A similar gradation was shown by the yield values of the plants from the non-treated pots; the yield in the case of soil R_1 being: 0.7 g, for soil R_2: 11.6 g, and for soil R_3: 45.9 g of grain per pot.

The yields from various treatments are summarized in Tables V and VI.

Table V, columns 4 and 5, show, in the case of soil R_1, rising increases in yield of both grain and straw for increasing applications of manganese sulphate and ferrous sulphate. These increases were more regular for grain than for straw. This agrees with previous findings that the yield of grain is more sensitive to manganese deficiency than is the yield of straw.

Soil R_2 (Table VI, columns 4 and 5) showed a large response in grain yields for manganese additions and a small response for

TABLE V

Dry matter yields and increases in yield and relative and absolute contents of manganese and iron, in oats following application of ferrous sulphate and manganese sulphate. Soil R_1 1952

Treatments	g dry matter per pot				ppm in dry matter				Uptake in mg/per pot			
	Yield		Increase in yield		Mn		Fe		Mn		Fe	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
1	2	3	4	5	6	7	8	9	10	11	12	13
1	0.7	25.7	—	—	15.0	19.6	137	514	0.01	0.50	0.10	13.21
f_1	0.8	28.2	0.1	2.5	9.5	17.7	132	440	0.01	0.50	0.11	12.41
f_2	1.3	28.5	0.6	2.8	10.2	17.6	109	400	0.01	0.50	0.14	11.40
f_3	2.1	36.7	1.4	11.0 †)	7.2	17.5	54	328	0.02	0.64	0.11	12.04
m_1	7.4	41.8	6.7 *)	16.1 †)	7.5	18.6	85	370	0.06	0.78	0.63	15.47
m_1f_1	11.6	49.1	10.9 †)	23.4 †)	6.6	16.5	79	453	0.08	0.81	0.92	22.24
m_1f_2	9.7	57.8	9.0 *)	32.1 †)	6.7	14.5	93	340	0.07	0.84	0.90	19.65
m_1f_3	13.1	48.7	12.4 †)	23.0 †)	7.1	14.0	78	323	0.09	0.68	1.02	15.73
m_2	13.9	40.2	13.2 †)	14.5 †)	7.2	17.5	69	353	0.10	0.70	0.96	14.19
m_2f_1	17.6	51.9	16.9 †)	26.2 †)	7.2	15.4	72	410	0.13	0.80	1.27	21.28
m_2f_2	18.0	51.7	17.3 †)	26.0 †)	7.7	14.4	76	300	0.14	0.74	1.37	15.51
m_2f_3	24.0	53.5	23.3 †)	27.8 †)	10.2	14.5	63	303	0.24	0.78	1.51	16.21
m_3	32.3	48.4	31.6 †)	22.7 †)	9.2	15.5	64	318	0.30	0.75	2.07	15.39
m_3f_1	41.5	50.8	40.8 †)	25.1 †)	11.8	12.4	74	280	0.49	0.63	3.07	14.22
m_3f_2	44.0	48.6	43.3 †)	22.9 †)	12.8	13.4	70	238	0.56	0.65	3.08	11.57
m_3f_3	47.8	50.0	47.1 †)	24.3 †)	13.4	13.3	60	330	0.64	0.67	2.87	16.50
S.E.	2.2	2.4	3.1	3.4								

*) 0.05 level; †) 0.01 level.

Plate I. Soil R_1 1952.

Plate II. Soil R_2 1952.

Plate III. Soil R_3 1952.

TABLE VI

Dry matter yields and increases in yield and relative and absolute contents of manganese and iron, in oats following application of ferrous sulphate and manganese sulphate. 1952

Treatments	g dry matter per pot				ppm in dry matter				Uptake in mg per pot			
	Yield		Increase in yield		Mn		Fe		Mn		Fe	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
1	2	3	4	5	6	7	8	9	10	11	12	13
Soil R_2												
1	11.6	39.8	—	—	9.0	14.3	108	390	0.10	0.57	1.25	15.52
f_2	14.4	44.7	2.8	4.9	7.5	12.3	102	265	0.11	0.55	1.47	11.85
m_2	34.1	42.5	22.5 †)	2.7	13.8	13.8	60	345	0.47	0.59	2.05	14.66
m_2f_2	36.1	41.1	24.5 †)	1.3	11.8	11.3	67	245	0.43	0.46	2.42	10.07
S.E.	2.5	2.8	3.5	4.0								
Soil R_3												
1	45.9	50.7	—	—	22.3	13.3	64	315	1.02	0.67	2.94	15.97
f_2	48.8	55.6	2.9	4.9 †)	20.4	12.3	62	318	1.00	0.68	3.03	17.68
m_2	49.0	54.6	3.1	3.9 *)	22.3	13.3	63	350	1.09	1.22	3.09	19.11
m_2f_2	50.8	60.3	4.9 *)	9.6 †)	26.4	14.9	63	333	1.34	0.90	3.20	20.08
S.E.	1.4	1.1	2.0	1.6								

*) 0.05 level; †) 0.01 level.

iron additions. The response of plants grown on soil R_3 was small for additions of both iron and manganese. On soils R_2 and R_3 the yields of straw showed only minor responses to the two treatments.

The relative contents of manganese and iron in the crops (ppm Mn and Fe in dry matter) are summarized in Tables V and VI, columns 6–9. The relative content of manganese in the grain from soil R_1 first declined and then rose again with increasing applications of manganese sulphate and ferrous sulphate. The relative content of manganese in the straw and the relative content of iron in both grain and straw generally decreased with increasing additions of manganese and iron.

On soils R_2 and R_3, the relative content of manganese in the grain decreased when iron alone was added to the soil and increased as a result of application of manganese or manganese together with iron. Variations in the relative manganese content of the straw as a result of addition of manganese and iron to the soils were small and uncertain.

The total manganese and iron contents of the crops per pot are summarized in Tables V and VI, columns 10–13. Steadily increasing total contents of manganese and iron in the grain in

response to increasing levels of manganese sulphate and ferrous sulphate, are shown in all three experiments. On the other hand, the increase in manganese and iron contents of the straw was more irregular.

b. Pot experiments 1953

The residual effect of ferrous sulphate was investigated in soil R_1 in 1953. As symptoms of magnanese deficiency were found in the crops grown at the highest manganese level in 1952, manganese sulphate was again applied in 1953 in the quantities shown in Table II. The experiment was extended by one more treatment in order to test the effect of ferric citrate. 1,000 mg iron as $FeC_6H_5O_7.3H_2O$ was added to five pots from a parallel experiment with soil R_1 in 1952 to which basal dressing had been applied in the amounts given in 1952. This treatment was designated f_2c. The design of experiment used for soil R_1 in 1952 — see Table II — therefore applies also in 1953 except that no ferrous sulphate was added in 1953 and the experiment was extended by one treatment, *viz.* f_2c.

Manganese sulphate and ferric citrate were supplied as solutions. Sejr oats were sown and the soil was kept at a moisture content equal to 40 per cent of its water holding capacity, calculated on an air-dry basis. The weights of the pots were recorded daily as in 1952.

A basal dressing of 7.5 g $NaNO_3$ and 5 g K_2HPO_4 were added in solution to each pot.

The oats were sown on 8/4. Germination began on 22/4 and was completed by 26/4. The first symptoms of manganese deficiency were observed on 18/5 in the non-treated pots. On 22/5 the plants were thinned to 25 plants per pot. Ear formation extended over the period 21/6–4/7. The crops grown at the highest manganese level produced ears first. Few and poor ears were formed in the non-treated pots.

In order to control aphids, the plants were sprayed 9 times during the growth period with a 1‰ nicotine solution. The harvest extended over a very long period, *viz.* from 30/7 to 5/9, the individual ears being harvested as they ripened. New basal shoots had not ripened at harvest.

In July, symptoms of manganese deficiency were observed in the oats of all the treatments. Iron deficiency was not observed during the growth period.

In view of the intensity and extent of the manganese deficiency the growth of the plants was assessed on July 14th. The scoring was in arbitrary units from 1–3, 1 representing the poorest and 3 the best growth. Average scores of 5 replicated pots are summarized in Table VII.

TABLE VII

Assessment of growth of oats on July 14th, 1953 Soil R_1	
Treatment	Mean scores *)
1	1.0
f_1	1.0
f_2	1.2
f_3	1.4
m_1	2.2
m_1f_1	1.8
m_1f_2	1.6
m_1f_3	2.0
m_2	2.6
m_2f_1	3.0
m_2f_2	3.0
m_2f_3	2.8
m_3	3.0
m_3f_1	3.0
m_3f_2	2.8
m_3f_3	3.0
f_2c	1.0

*) Scoring in arbitrary units: 1 = poor growth; 3 = best growth.

Table VII shows that addition of ferric citrate did not affect the growth. The assessment also showed that better growth was due mainly to the application of manganese.

The yield values are shown in Table VIII.

From the increase in yields shown in columns 4 and 5 it will be seen that the addition of ferric citrate did not produce higher yields in either grain or straw, and also that the increase in yields was due to the manganese sulphate added and not to a residual effect of ferrous sulphate. This is shown even more clearly in Table IX where the effect of iron on soil R_1 in 1952 and 1953 is shown as averages of the four manganese levels.

It will be seen that the effect of iron found in 1952 was not observed again in 1953.

The relative content of manganese in the grain in 1953 (Table VIII, column 6) shows – as in 1952 — first a decline and then an

TABLE VIII

Dry matter yields and increases in yield and relative and absolute contents of manganese and iron, in oats following application of ferrous sulphate and manganese sulphate
Soil R_1 1953

Treatments	g dry matter per pot				ppm in dry matter				Uptake in mg per pot			
	Yield		Increase in yield		Mn		Fe		Mn		Fe	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
1	2	3	4	5	6	7	8	9	10	11	12	13
1	5.5	17.2	—	—	11.9	20.3	79	688	0.07	0.35	0.43	11.83
f_1	6.9	24.7	1.4	7.5 *)	11.0	19.1	80	675	0.08	0.47	0.55	16.67
f_2	11.0	26.6	5.5	9.4 *)	11.1	17.8	81	510	0.12	0.47	0.89	13.57
f_3	8.8	21.9	3.3	4.7	9.0	19.0	83	596	0.08	0.42	0.73	13.05
m_1	27.5	37.4	22.0 †)	20.2 †)	11.5	16.1	69	537	0.32	0.60	1.90	20.08
m_1f_1	22.3	34.8	16.8 †)	17.6 †)	12.2	14.7	81	436	0.27	0.51	1.81	15.17
m_1f_2	20.5	28.2	15.0 †)	11.0 †)	11.4	15.6	82	471	0.23	0.44	1.68	13.28
m_1f_3	22.5	28.8	17.0 †)	11.6 †)	12.4	18.8	72	446	0.28	0.54	1.62	12.85
m_2	31.7	34.6	26.2 †)	17.4 †)	13.2	13.9	67	392	0.42	0.48	2.12	13.56
m_2f_1	33.5	35.4	28.0 †)	18.2 †)	13.7	15.5	74	401	0.46	0.55	2.48	14.20
m_2f_2	39.4	34.8	33.9 †)	17.6 †)	12.0	15.1	73	403	0.47	0.53	2.88	14.02
m_2f_3	30.7	29.7	25.2 †)	12.5 †)	13.1	18.6	74	413	0.40	0.55	2.27	12.27
m_3	39.6	39.9	34.1 †)	22.7 †)	18.4	12.6	63	365	0.73	0.50	2.49	14.56
m_3f_1	35.3	36.0	29.8 †)	18.8 †)	21.7	18.7	70	407	0.77	0.67	2.47	14.65
m_3f_2	35.9	33.9	30.4 †)	16.7 †)	20.1	19.4	79	342	0.72	0.66	2.84	11.59
m_3f_3	33.0	30.7	27.5 †)	13.5 †)	23.1	21.5	75	379	0.76	0.66	2.48	11.64
f_2c	4.8	16.9	–0.7	–0.3	10.9	19.5	72	591	0.05	0.33	0.35	9.99
S.E.	2.9	2.3	4.1	3.3								

*) 0.05 level; †) 0.01 level.

TABLE IX

Effects of iron. Mean values
Soil R_1 1952 and 1953

Year	Grain g/pot			Straw g/pot		
	F_1	F_2	F_3	F_1	F_2	F_3
1952	4.5	4.9	8.4	12.4	14.1	14.6
1953	—1.6	0.6	—2.3	0.5	—1.4	—4.5

increase with the rising additions of manganese sulphate and ferrous sulphate. However, the figures in column 6 reveal that these trends in relative manganese contents were due only to the manganese sulphate added, because no regular decrease or increase was found at different manganese levels as a result of the ferrous sulphate added in 1952. The relative manganese content of the straw and the relative iron content of the grain and the straw decreased with rising supplies of manganese.

Total manganese contents of the crop per pot (columns 10 and 11) increased with increasing supplies of manganese. No residual effect of the ferrous sulphate added in 1952 was found in this case either.

DISCUSSION AND CONCLUSION

Results from previous experiments [1] suggested that application of ferrous sulphate may have the same effect as application of manganese, since an addition of ferrous sulphate increased the uptake of manganese by plants (barley and sugar beet) on two clay soils and one muck soil in pot experiments. Addition of ferrous sulphate to a muck soil deficient in manganese was also seen to produce a significant increase in the yield of sugar beet, both roots and tops, when adequate nitrogen was applied. Symptoms of iron deficiency were not observed, either in the experiments made in 1950 and 1951 [1] or in the pot experiments with oats in 1952 and 1953 reported here.

The pot experiments in 1952 were carried out with two sandy soils and one moor soil, all having low contents of exchangeable manganese as determined by exchange with 1 *M* $Mg(NO_3)_2$ (Table I). Oat crops on the two sandy soils showed severe and widespread symptoms of manganese deficiency; such symptoms were few and sparse in the moor soil R_3 and the deficiency was of no significance to the growth of the oats (Table IV).

Since the effect of manganese on the nutrition of cereals is most marked, both quantitatively and qualitatively, in relation to yield of grain only the results relating to grain yields will be dealt with here, and only with reference to soil R_1.

Table X shows the effects which are due only to supplies of ferrous sulphate (F_1 — F_3) at different levels of manganese (m_1 — m_3), the effect of manganese sulphate having been deducted in the treatments where both manganese sulphate and ferrous sulphate were applied.

As will be seen from Table X, the increase in grain yield and manganese uptake, following application of increasing quantities of ferrous sulphate at different manganese levels, is progressive.

Symptoms of iron deficiency were not observed during the growth period, and since addition of ferric citrate to the soil in 1953

TABLE X

Effect of increasing applications of ferrous sulphate on grain yield and manganese content of oats at four different manganese levels
Soil R_1 1952

Mn added	Increase in grain yield g dry matter per pot			Increase in uptake mg manganese per pot		
	F_1	F_2	F_3	F_1	F_2	F_3
0	0.8	1.3	2.1	0	0	0.01
m_1	4.2	2.3	5.7	0.02	0.01	0.03
m_2	3.7	4.1	10.1	0.03	0.04	0.14
m_3	9.2	11.7	15.5	0.19	0.26	0.34
Average	4.5	4.9	8.4	0.06	0.08	0.13

(Table VIII) had no effect on the yield or on the manganese content of the grain, it must be supposed that the effect observed from ferrous sulphate application could not be due to an elimination of iron deficiency. Also the addition of ferrous sulphate had the same effect, on this sandy soil, as an application of manganese sulphate. If the effect of ferrous sulphate is through a manganese effect the shape of the yield curve describing the relationship between absorbed manganese and the dry matter yield per pot due to application of ferrous sulphate ought not be different from the curve which represents the same relationship due to application of manganese sulphate.

In Fig. 1 the curve drawn as a continuous line through four points represents the relationship between absorbed manganese and yield of dry matter per pot due to the application of manganese alone. This S-shaped curve shows that soil R_1 is very manganese deficient (Steenbjerg [13]). The curve describing the relationship between the manganese content of the dry matter and the yield of dry matter per pot due to application of increasing quantities of ferrous sulphate, either alone or together with increasing quantities of manganese sulphate, coincides with the above mentioned curve or takes the same form (broken line). Hence, it must be concluded that the effects of manganese sulphate and of ferrous sulphate were of the same nature in 1952. In 1953 the residual effect did not give rise to any increase in yield (Table VIII) as a result of the ferrous sulphate applied in the previous year.

It is well-known that the higher manganese oxides of soils may become important for the nutrition of plants, cf. Steenbjerg [9]; Leeper [5 6]; Jones and Leeper [3 4]. Steenbjerg

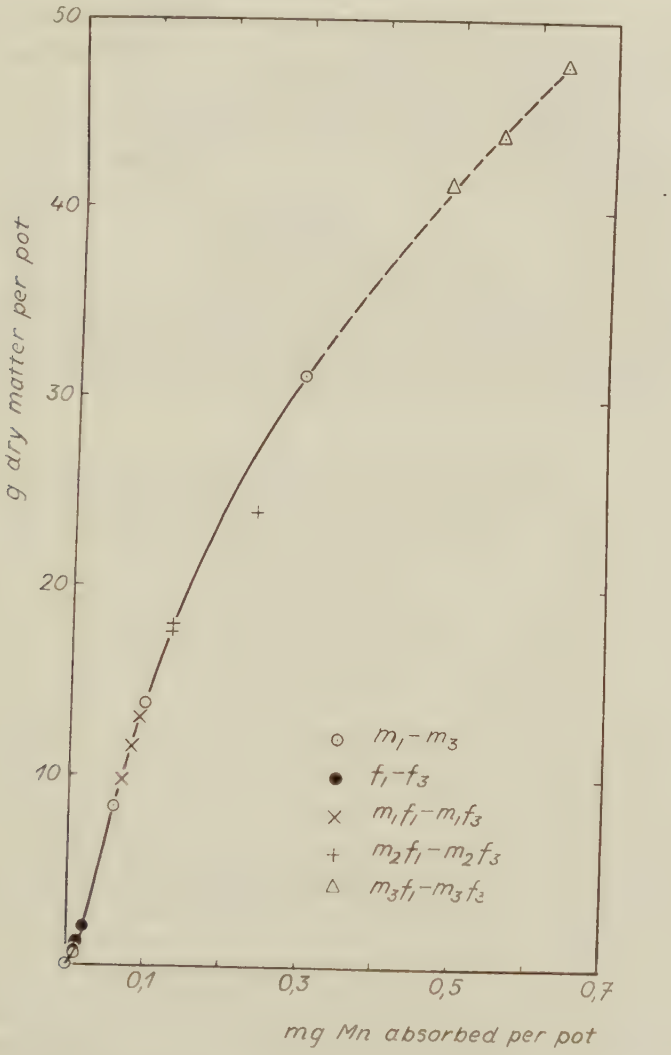

Fig 1. Relationship between manganese uptake and dry matter yield (grams per pot for oats growing on soil R_1 1952.

demonstrated inter alia that pyrolusite was available to the plants as a source of manganese when reducing agents, such as hydroquinone, are added to the soil.

Since ferrous sulphate can act as a reducing agent in alkaline solutions (M ü l l e r [7]) it is natural to suppose that the manganese

effect, observed in the pot experiments on addition of ferrous sulphate, is due mainly to the release of available manganese by reduction of higher manganese oxides.

If the manganese effect of ferrous sulphate is due to reduction of higher manganese oxides, a similar manganese effect should be found by using a reducing agent such as hydroquinone. On this assumption, laboratory investigations were made with the three soils used for the pot experiments in order to ascertain whether manganese is released in the same quantities when the soil is treated with equivalent quantities of either ferrous sulphate or hydroquinone. The procedure described under the heading of "Methods" was used, and the results are shown in Table XI.

TABLE XI

Amount of reducible manganese extracted by *M* magnesium nitrate containing equivalent quantities of hydroquinone and of ferrous sulphate

Soil	ppm Mn	
	Hydroquinone	Ferrous sulphate
R_1	19.2	20.8
R_2	26.0	19.0
R_3	28.0	28.0

It will be seen that the quantities of manganese released from the soils treated with equivalent amounts of ferrous sulphate and of hydroquinone are of the same order. This strengthens the assumption that the manganese effect of ferrous sulphate was due to the ability of the latter to reduce higher manganese oxides. This agrees with the observation that in the pot experiments no residual effect of ferrous sulphate added in 1952 was found in the oats grown in 1953 which may be ascribed to the reducing effect of ferrous sulphate probably being of short duration in alkaline soils.

Table X also shows that at all three levels of application of iron there were rising increases in yields with increasing manganese levels. This suggests either that addition of ferrous sulphate prevents the transformation of manganese sulphate to less available compounds, (Heintze and Mann[2]), or that the recently precipitated compounds in the manganese treated soils are reduced more easily than the older precipitated manganese compounds in the non-treated pots. A combination of the alternatives may perhaps also occur.

Thus, from an agricultural point of view, it is possible to relieve manganese deficiency in soils containing considerable quantities of reduceable manganese by applying ferrous sulphate. Such a procedure however, depletes the supplies of this nutrient in the soil and consequently it can only be used advantageously for a limited period.

SUMMARY

Pot experiments with oats on manganese deficient sandy and moor soils, which are not deficient in iron, showed a steady increase in yield and manganese uptake by the plants with increasing additions of ferrous sulphate at four levels of manganese. Residual effects from ferrous sulphate application were not found either in the case of yields or manganese uptake by oats on a sandy soil.

The effect observed following application of ferrous sulphate was due to a manganese effect. This is corroborated by the coincidence of the yield curves, showing the relationship between absorbed manganese and yield of dry matter, following the application of manganese sulphate and ferrous sulphate.

Soils treated with *M* magnesium nitrate in the presence of equivalent quantities of either ferrous sulphate or hydroquinone yielded the same amounts of manganese. This result suggests that the manganese effect of ferrous sulphate is due to reduction of higher manganese oxides by ferrous sulphate.

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STUDIES ON THE DECOMPOSITION OF AMINO ACIDS IN SOILS

I. A PRELIMINARY SURVEY OF TECHNIQUES

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INTRODUCTION

The decomposition of protein is a process that must take place continuously in all soils: cells of plants and cells of micro- and macro-organisms are always dying and autolyzing, the proteins resulting from the autolysis are likewise always being decomposed by the soil micro-organic population. Almost certainly the first stage of protein decomposition in soil must be the hydrolysis of the protein into its constituent amino acids, or at least into rather simple peptides. A knowledge of the kinetics and mechanism of amino acid decomposition in soils is therefore of considerable importance to a proper understanding of protein decomposition. And yet, rather surprisingly, there seems to have been little really concentrated investigation of this problem apart from some important recent work by Tombesi [11]. In this paper, therefore, we shall present some results on amino acid decomposition in soil that have been obtained by two modifications of the soil percolation technique. For reasons that will become apparent, we believe that these two modifications are particularly suitable for such studies; we shall describe them in detail here since we hope to use them in an extensive survey of amino acid decomposition in different types of soil.

TECHNIQUES

If serine is taken as an example, the complete aerobic breakdown of an amino acid in soil may be represented as:

$$CH_2(OH).CH(NH_2).COOH + 2\tfrac{1}{2}O_2 \quad 3\,CO_2 + 2\,H_2O + NH_3 \quad (1)$$

possibly followed by nitrification of the ammonia:

$$NH_3 + 2\,O_2 = HNO_3 + H_2O \qquad (2)$$

These equations indicate that the aerobic breakdown of an amino acid in soil might be followed in three ways (*a*) by measurement of the amount of oxygen consumed in the breakdown, due allowance being made for any oxygen consumed in reaction (*2*); (*b*) by measurement of the amount of carbon dioxide liberated; (*c*) by measurement of the amount of nitrogen mineralized. In the present work we have used all three types of measurement to study the breakdown in soil of 17 common amino acids. Two different percolation techniques have been employed:

1) The standard soil percolation technique adapted to the estimation of carbon dioxide production. This technique has already been described [2,4]; no modification of apparatus or operation has been found necessary. The apparatus of this technique we now refer to as the 'forced air percolator.'

2) The 'percolating respirometer' technique which measures the oxygen uptake of a soil/percolate system metabolizing some oxidizible compound. This technique has already been briefly described [3], but since that description the apparatus has been somewhat simplified and its operation made more convenient. The modified percolator is shown in Figure 1; we now refer to this apparatus as the 'rocking percolator'. The main differences between the apparatus now in use and that originally described [3] are:

a) The alkali cup is now sited in the percolator cap (P.C., Fig. 1) instead of being inserted into the soil itself. The cups now in use (A.C. Fig. 1) are 10-ml polythene specimen tubes with integral hinged lids. These tubes, containing 3 ml 40% NaOH into which dips a roll of Whatman 54 filter paper, are inserted, with the hinged lids sprung backwards, into the percolator caps P.C. at the start of an experiment. The resilience of the hinge presses the polythene lid against the side of P.C. and so retains the tube A.C. in position. The tubes are easily removed at the end of the experiment for cleaning and re-filling.

b) A small polythene disk (P.D. Fig. 1), drilled with about a dozen 0.5-mm holes, is used instead of a glass wool plug to retain the soil in position; this modification maximizes speed of percolation and minimizes clogging.

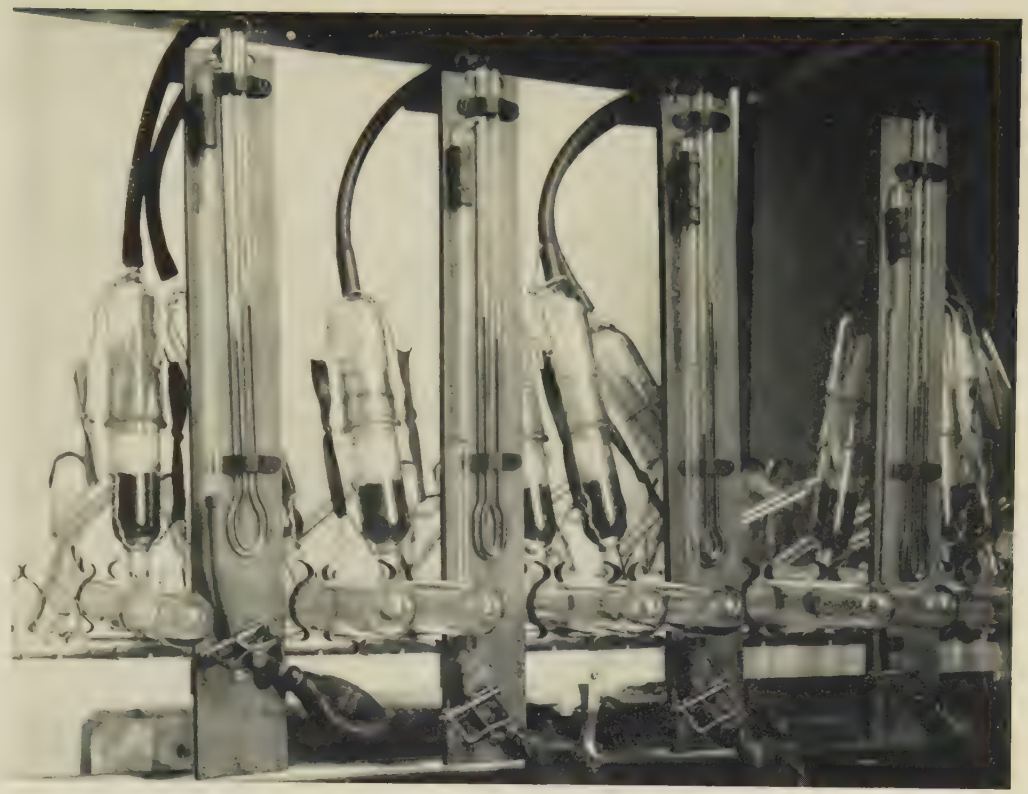

Plate I. The rocking percolator assembly.

c) The manometer and 5-ml compensating pipette have been modified and are now kept permanently connected to the percolator caps by suitable lengths of heavy gauge pressure tubing. The whole assembly — manometer (M) pipette (P) and percolator caps (P.C.) — is permanently fixed to a wooden board mounted on the rocking table of the apparatus. The assembly is shown in Figure 2. Water is introduced into, or removed from, the pipette P, by manipulation

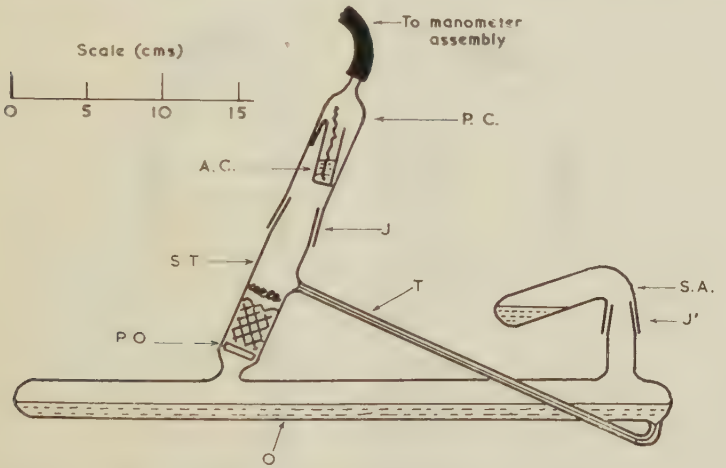

Fig. 1. The rocking percolator. J = B29 joint; J′ = B19 joint; S.T. = soil tube; S.A. = side arm; P.C. = percolator cap permanently connected to manometer assembly (Fig. 2); A.C. = 10–ml polythene specimen tube containing caustic soda and a roll of hardened filter paper; P.D. = polythene disk drilled with 0.5–mm holes. The transfer tube T is 2–3 mm capillary. The apparatus is rocked between the horizontal and 60° to the horizontal round a fulcrum roughly at 0.

of the water-filled rubber bulb (R.B.) and screw clip (S.C.). A complete rocking percolator assembly mounted on its table is shown in Plate I.

Operation of the rocking percolators

At the start of the experiment, 10 g of sieved soil is placed in the soil tube of each of a pair of percolators and 45 ml of water in each main tube. The percolators are then clipped onto the rocking table of the apparatus and the percolator caps P.C. fitted. It is essential to be sure that the B29 glass joint J (Fig. 1) is well greased and free from grit. Rubber retaining bands, not shown in Fig. 1, are stretched

between lugs on the caps P.C. and lugs on the soil tube S.T. These bands hold P.C. in position and obviate gas leakage at the joint J. The mechanical rocking of the table (at about 2 cycles/min.) is now begun. During an initial equilibration period of some 4 days, one percolator may take up a little more, or a little less, oxygen than its companion; consequently the level of the manometer fluid in M (Fig. 2) may show some fluctuation. After about 4 days, however,

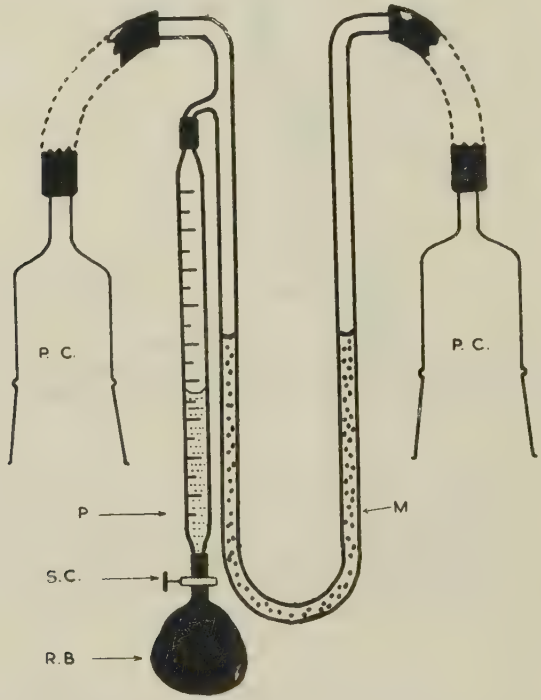

Fig. 2. The manometer assembly. R.B. = 20–ml rubber bulb filled with water; S.C. = screw clip; P = 5-ml pipette; M = manometer; P.C. = percolator caps of Fig. 1.

equilibration will probably be complete and thenceforward the level of the manometer fluid will remain steady. After equilibration has been attained, the side arms S.A. (Fig. 1) are removed from both percolators; 5 ml of amino acid solution containing 10–50 mg of amino acid, is placed in the left hand side arm and 5 ml water in the right hand side arm. The screw clip S.C. (Fig. 2) is released so that *almost* all the water in pipette P (Fig. 2) runs into the rubber bulb

R.B. attached to the pipette. When the water in P has attained *almost* its lowest level, S.C. is closed and the side arms replaced on their B19 joints (J', Fig. 1) which should, of course, be well greased to obviate gas leakage. Rocking of the apparatus is then re-started and continued for about 1 hour in order to even out temperature differences that may have been set up between the two paired percolators as a result of handling the side arms. The clip S.C. is then released again and the amount of water in P is adjusted until the level of the manometer fluid is the same on both sides of the manometer M. The clip S.C. is then closed and the pipette reading of the water in P noted; the side arms are turned through 180° in order to tip the amino acid solution into the main percolate [3] and rocking is begun once more. The left hand percolator, containing amino acid solution, should begin to consume oxygen more rapidly than its companion containing only water. The manometer fluid therefore rises on the left hand side; it may be relevelled at any time by forcing water into P from R.B. The volume of water that must be forced into P to bring about relevelling is an exact measure of the volume of oxygen consumed in the left hand percolator (as a result of amino acid decomposition) over and above that consumed in the right hand percolator (as a result of general soil metabolism). When the differential oxygen uptake on the left hand side has exceeded 5 ml (the total capacity of P), the level of water in P is lowered *almost* to its limit and both side arms S.A. removed simultaneously to effect re-equilibration of gas pressure between the companion percolators. The side arms are then replaced, and the experiment continued as though it had just begun.

By means of this technique it is possible to follow with reasonable accuracy ($\pm$ 0.05 ml) the oxygen uptake consequent upon the percolation of a soil by a solution of a known amount of an amino acid. Since it is normally the O_2 consumed according to Eqn. (*1*) that is of interest, the oxygen uptake resulting from nitrification of released ammonia (Eqn. (*2*)) must be compensated for. From time to time, therefore, the percolates are sampled and their nitrate concentrations determined. From these determinations it is possible to calculate the amount of oxygen consumed as a result of nitrification (Eqn. (*2*)). Subtraction of this from the total amount of oxygen consumed give the amount of oxygen consumed as a result of the oxidative breakdown of the amino acid according to Eqn. (*1*).

MATERIAL AND METHODS

Soil

The soil on which the bulk of the present experiments were carried out was a sandy loam from an Aberdeen garden, pH 6.5. Before use, it was air dried and sieved between the limits 4.0 mm–1.0 mm. To maximize the stability of the soil crumbs under percolation conditions we have always added soil conditioner ("Krilium") to the percolates at the rate of about 1 mg/100 ml. To minimize possible biological changes in the soil as a result of long-term storage, we have always used our soil within four weeks of its being brought into the laboratory.

Ammonia-N

Ammonia-N was determined on 1-ml samples of percolate by the Conway technique [1],

Nitrite- and nitrate-N

Nitrite and nitrate-N were determined as previously described [5] on 1–ml samples of percolate. Nitrite-N was always less then 1.0 μg/ml.

Amino-N

Amino-N was determined on 1–ml samples of percolate by a micro-modification of the method of Pope and Stevens [7]. This determination was not, however, carried out on every sample. Where it was not carried out, the concentration of amino-N in a sample was estimated by visual inspection of colour density of the ninhydrin spot given by the amino acid after paper chromatography of the sample (see below). The estimation of amino-N concentration by this method was accurate to about 20%; this accuracy was sufficient for most of the present work.

Chromatography

Every sample of percolate taken was run on a one-dimensional paper chromatogram with water/butanol/acetic-acid (5 : 4 : 1) as solvent; the spots were developed by a ninhydrin/butanol/collidine spray. All amino acid percolations were run at least in duplicate and in at least one of the replicated experiments, two additional chromatograms were performed on every sample of percolate. These two additional one-dimensional chromatograms were run with phenol/water and collidine/water as solvents.

Sampling of the percolators

If the percolators are sampled during the course of breakdown of an amino acid, the nitrogen mineralized, the oxygen consumed, and the carbon dioxide evolved, will always be less than 'theoretical' values derived from Eqn. (*1*) by amounts corresponding to the total amounts of material (amino acid, ammonia, nitrate *etc.*) removed during sampling. It is therefore necessary,

under these circumstances, to correct the observed values of nitrogen mineralization, oxygen uptake, or carbon dioxide evolution, for the effects of sampling. We have based our sampling corrections on the assumption that the organic acids resulting from deamination of the initial amino acid were not adsorbed at all by the soil, and on the observation that, under our conditions of experiment, the amino acids were not appreciably adsorbed by the soil. We have checked the validity of these sampling corrections by running parallel experiments in the rocking percolator with the same amount of amino acid in two rocking percolator assemblies. From one of these assemblies five samples of 5 ml each were withdrawn during the course of the experiment, from the other no sample was withdrawn. The corrected oxygen uptake of the first assembly agreed within 5% with the oxygen uptake observed in the second assembly. The corrections applied therefore had experimental validation; all figures quoted have been corrected for sampling.

Experimental conditions

Experiments with the forced air percolators were all carried out with 10 g soil and 100 ml percolate containing 100 mg amino acid. Experiments with the rocking percolators were all carried out with 10 g soil and 50 ml percolate, containing 10–50 mg amino acid. All experiments were run in the dark at 20°C.

Adjustment of pH

A few experiments were run at pH values different from the natural pH of the soil used (6.5). In these experiments the soil was first percolated for about a week with $M/20$ phosphate buffer with an initial pH value of 2.6, 6.5, or 8.2. After this 7-day period, pH drift in the percolate had ceased and the actual pH value of the percolate was determined. The amino acid was then added to the percolate and the experiment proper begun. The actual pH values at which these experiments were run, *i.e.* the pH values of the percolates determined just prior to the addition of the amino acid, were 3.8, 6.5 and 8.0.

RESULTS

For most of the amino acids studied, the pattern of breakdown was very similar to that shown by DL-serine. The O_2-uptake, mineralization of nitrogen, disappearance of amino-N, and the O_2-uptake due to nitrification of ammonia, observed when 45 mg serine was used in the rocking percolator, are shown in Fig. 3. The O_2-uptake curve corrected for the O_2-uptake due to nitrification of ammonia is shown in Fig. 4, together with the CO_2-output curve for 100 mg DL-serine studied in the forced air percolator. The ordinate of this graph is the percentage of the 'theoretical' O_2-uptake or CO_2-output according to Eqn. (*1*); the use of this ordinate enables valid compari-

sons of CO_2-output and O_2-uptake to be made. The two curves of Fig. 4 are very similar, despite the fact that different soil samples were used and despite the fact that the amount of amino acid used to obtain the CO_2-output curve (100 mg) was more than twice that used to obtain the O_2-uptake curve (45 mg). Similarly good agreement between the CO_2-output curve and the O_2-uptake curve has been found with all the other amino acids (except cystine) even when the amount of amino acid used in the rocking percolator was only 10 mg, *i.e.* only one-tenth of that used in the forced air percolator. The results with cystine in the rocking percolator were anomalous because of O_2-uptake due to sulphide oxidation.

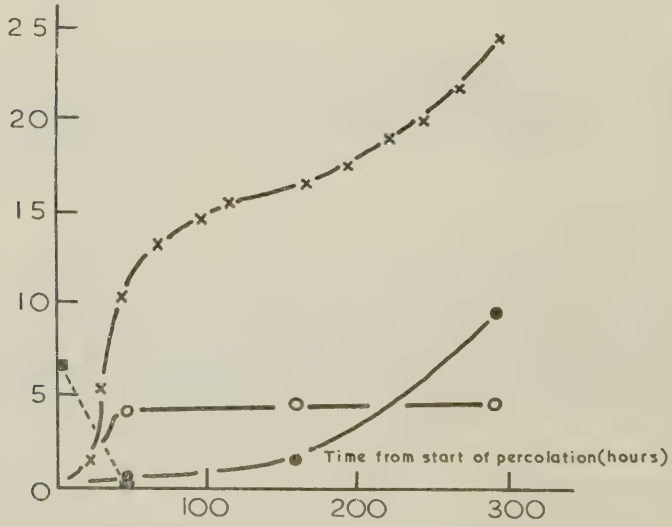

Fig. 3. The breakdown of 45 mg DL-serine in the rocking percolator. Soil wt. = 10 g; percolate volume = 50 ml. - - - ■ - - - total amino N in percolator (mg). —×— O_2-uptake observed (ml). —•— O_2-uptake due to nitrification of ammonia (ml) (calculated from nitrate formation in percolate). — ○ — total mineral N in percolator (mg). All figures have been corrected for sampling errors.

It will also be noted that, according to neither curve was serine decomposition complete. Yet, according to amino-N determinations on the percolates and according to the evidence provided by paper chromatograms of the percolates, all the serine had been destroyed and no other amino acid had been formed. Nor was the amount of N

appearing as mineralized-N (ammonia + nitrite + nitrate) equal to the amount of amino-N disappearing. These observations merely show that, as might be expected, part of the serine initially added to the percolate had been built up by the micro-organic population of the soil into nitrogen-containing organic material. It is possible to calculate from (*a*) the amount of O_2 consumed or the amount of CO_2 evolved and (*b*) the amount of nitrogen mineralized, the C : N ratio of the organic material residual in the soil after the percolation

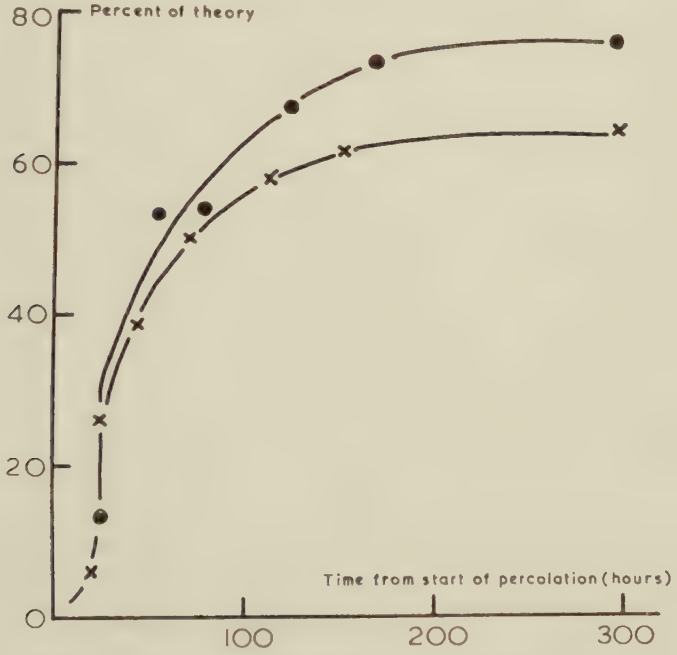

Fig. 4. The O_2-uptake of, and CO_2-output from, DL-serine percolated through soil. —×—, O_2-uptake, corrected for uptake due to nitrification, for the results shown in Fig. 3. —•— CO_2-output from 100 mg serine percolated in 100 ml percolate through 10 g soil in forced air percolator. O_2-uptake and CO_2-output are shown as percentage of theory according to Eqn. (*1*).

and breakdown of a known amount of a given amino acid. In Table I are shown the values of O_2-uptake, CO_2-output, and N mineralization, for all the common amino acids; these figures are expressed as 'percentages of theoretical' on the analogy of Eqn. (*1*). In Table II are shown the C : N ratios, calculated from the data of Table I, of

TABLE I

The O_2-uptake, CO_2-output, and N-mineralization, values for all the common amino acids percolated through garden soil.

All values are calculated as 'percentage of theory' on the analogy of Eqn. (*1*). All values were obtained with 10 g soil sieved 4.0–1.0 mm. Values for O_2-uptake were obtained with 50 ml percolate and 10–50 mg amino acid, values for CO_2-output were obtained with 100 ml percolate and 100 mg amino acid. Values for N-mineralization have been calculated as the mean of those observed in the rocking percolator and those observed in the forced air percolator

Amino acid	CO_2-output	O_2-uptake	N-mineralization
DL-alanine	64	60	57
L-arginine	71	73	70
L-aspartic acid	68	70	68
L-cystine	80	†	67
L-glutamic acid	61	66	60
Glycine	69	70	77
L-histidine	57	60	64
L-leucine	56	58	34
L-lysine	66	74	69
L- and DL-methionine *	—	—	—
L-phenylalanine	74	77	34
L-proline	70	56	51
DL-serine	78	66	72
L- and DL-threonine *	48	39	34
L-tryptophan	65	67	40
L-tyrosine	70	66	22
L- and DL-valine	70	60	45

* Results with these amino acids showed anomalies; see Discussion.

† This value could not be calculated because there was an extra O_2-uptake due to the oxidation of the SH′, derived from the cystine.

the organic materials residual in the soil after percolation of the different amino acids; for the purposes of Table II, the extent to which the carbonaceous moiety of the amino acid was oxidized to CO_2 has been taken as the mean of the extent indicated by O_2-uptake and the extent indicated by CO_2-output.

Lees and Porteous[4] found that when glucose was percolated through two tropical soils there was first of all a very short lag phase, after which CO_2 was released from the glucose according to the equation:

$$\log_{10}\frac{C}{C-c} = K.t$$

Where C = total amount of carbon finally converted to CO_2
c = amount of carbon converted to CO_2 at time "t"
K = a constant.

TABLE II

The C : N ratios of the organic materials residual in the soil after percolation of different amino acids

The figures of this Table are calculated from those of Table I. The values for carbon percolated but not oxidized to CO_2, on which the figures given below are calculated, have been derived from the mean values of carbon oxidized to CO_2 according to O_2-uptake and CO_2-output shown in Table I

Amino acid	C : N ratio of residual material *
DL-alanine	2.3
L-arginine	1.2
L-aspartic acid	3.3
L-cystine	1.6
L-glutamic acid	3.9
Glycine	2.2
L-histidine	2.0
L-leucine	3.4
L-lysine	2.3
L-phenylalanine	2.8
L-proline	3.3
DL-serine	2.8
DL-threonine	3.1
L-tryptophan	2.7
L-tyrosine	3.3
DL-valine	2.8
Mean	2.7

* The ratio "g C : g N".

Similar calculations have been carried out on the CO_2-output and O_2-uptake curves shown in Fig. 4. In these calculations it was assumed that the total amount of carbon (C) finally converted to CO_2 was 80% of theory; the "C" value for O_2-uptake was assumed to be 68% of theory. The resultant graph is shown in Fig. 5. The K-value for this graph turns out to be $1.8 \times 10^{-6} \times \text{seconds}^{-1}$ compared with the value of $1.2 \times 10^{-6} \times \text{seconds}^{-1}$ for glucose decomposition in tropical soils. We have carried out similar calculations for all the amino acids studied. All the K-values lie between 1.0×10^{-6} and $3.0 \times 10^{-6} \times \text{seconds}^{-1}$ except for threonine and methionine. For these amino acids, which show abnormal kinetics, K-values cannot be validly calculated.

The effects of changing the pH of the percolate on O_2-uptake and CO_2-output have been studied for a number of amino acids (leucine, lysine, arginine, and glutamic acid). The only marked effect of pH

change was that at low pH values there was a delay before amino acid decomposition began. Typical results (for the O_2-uptake of lysine percolated at different pH values) are shown in Fig. 6.

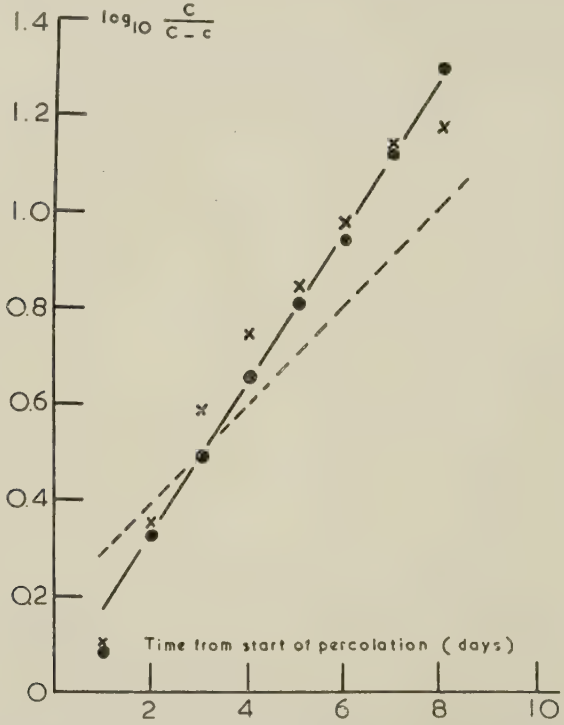

Fig. 5. O_2-uptake of, and CO_2-output from, DL-serine percolated through soil. C = final O_2-uptake or CO_2-output, c = O_2-uptake or CO_2-output at the indicated time. Dotted line is the corresponding line for the CO_2-output from glucose percolated through tropical soils [4]. —×— = O_2-uptake, —●— = CO_2-output.

DISCUSSION

The decomposition of all the common amino acids in a garden soil has been followed by two different percolation techniques. In one of these techniques, the O_2-uptake consequent upon decomposition of the amino acid was measured, in the other, the CO_2-output resulting from decomposition. In both technique measurements were made of the nitrogen released into the percolate as mineral nitrogen (ammonia, nitrite, nitrate) as a result of amino acid decomposition.

There was both qualitative and quantitative agreement between the results obtained by the two different techniques.

Most of the common amino acids (the exceptions being threonine and methionine) decomposed in rather similar ways. There was a very rapid deamination (usually complete in 24–36 hours) and an almost equally rapid initial CO_2-release and O_2-uptake consequent upon the decomposition of the carbonaceous moiety resulting from deamination of the original amino acid. It is interesting to note that the kinetics of CO_2-release (and O_2-uptake) during the decomposition of a typical amino acid (DL-serine) in the garden soil (Fig. 5) were

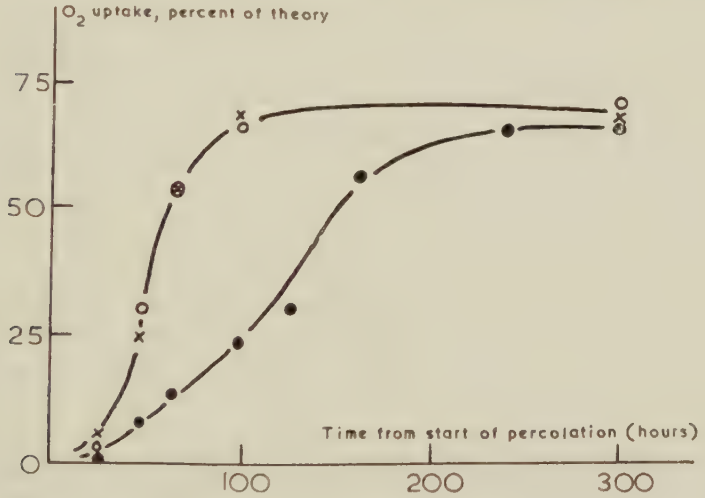

Fig. 6. O_2-uptake of 15 mg L-lysine percolated in 50 ml percolate through 10 g of the same garden soil held at different pH values by *M* 20 phosphate buffer in the percolate. —•— pH 3.8, —○— pH 6.5, —×— pH 8.0.

similar to those of the decomposition of glucose in the 'crumb layers' of two tropical soils [1]. It seems possible that the '*K* values' obtained in the ways used to construct the graph of Fig. 5 may prove to be useful numerical guides in the comparison of the rate of breakdown of the same compound in different soils or the rate of breakdown of different compounds in the same soil.

During the decomposition of the amino acids in the garden soil, some of the carbon and nitrogen initially present in the amino acid were retained by the soil, presumably as temporary augmentations of the organic compounds composing, or elaborated by, the micro-

organic population of the soil. This is a well recognised phenomenon that has recently been investigated by other workers [6 8 9]. We have made calculations (Table II) (on the bases of the amounts of nitrogen initially present in the percolated amino acid but *not* mineralized, and the amounts of carbon initially present in the amino acid but *not* converted to CO_2) of the C : N ratio of the organic material temporarily "locked up" in the soil as a result of amino acid breakdown. These calculations cannot, in the nature of things, be very accurate, since some 70% of the carbon of the amino acids was released as CO_2 and a similar percentage of the initially organic nitrogen was mineralized; a small percentage error in the estimation of these quantities would therefore result in a considerably larger percentage in the estimate of the 'residual' C : N ratio. Nevertheless, the C : N ratios calculated in this way mostly lay between 2.0 and 4.0. This suggests that the bulk of the material 'locked up' as a result of amino acid decomposition in a soil had a C : N ratio not dissimilar from that of a normal protein (C : N = 3.5 approx.). The locked-up material may therefore well have been mainly proteinaceous in nature.

Despite the fact that the decomposition of most of the amino acids conformed to a generally similar pattern, two amino acids (L- and DL-threonine and L- and DL-methionine) behaved anomalously.

The decomposition of L- or DL-threonine was very slow according to O_2-uptake, CO_2-output, and N-mineralization, criteria. Paper chromatograms carried out after eight days percolation of threonine showed the presence of appreciable quantities of threonine. The reason for this slow decomposition of threonine is not known. It may be that threonine deaminase is, for most soil organisms, an adaptive enzyme whose elaboration is a lengthy process. The problem is now under investigation.

The behaviour of L- or DL-methionine was completely anomalous. De-thiolation of some of the methionine, as detected by the odour of mercaptans issuing from the forecd air percolators, was rapidly established. Yet, as judged by the intensity of the methionine spot on paper chromatograms and by the O_2-uptake or CO_2-output of soils percolated with methionine, decomposition of the rest of the methionine was always slow. There was the further complication, too, that the rate of methionine decomposition, although always slow

compared with the rate of decomposition of a "normal" amino acid such as leucine, nevertheless varied markedly from one soil sample to another. This variation occurred despite the fact that the soil samples were all taken from the same area of the same garden. Starkey, Segal, and Manaker[10] observed that many micro-organisms grew well on methionine only when additional organic compounds were present in the medium. Preliminary work we have carried out suggests that methionine decomposes more rapidly in soils if glucose is added to the soil along with the methionine. We are pursuing investigations of this kind in the hope that they will provide some explanation of the somewhat discordant results noted above.

Although the results here presented are meant to show the potentialities of the techniques used rather than to establish a pattern of "amino acid metabolism in soil" they bring out one point very clearly. They show that measurement of the CO_2 evolved or of the O_2 consumed during the decomposition of a nitrogenous material in soil are better methods of following the decomposition than measurement of the rate at which nitrogen is mineralized. Because the C : N ratio of the organic material 'locked up' as a result of amino acid decomposition in soil is always apparently about 3 : 1, the C : N ratio of the material added to the soil has a considerable effect *per se* on the percentage of nitrogen mineralized during decomposition of the material. This effect has, of course, been found by other workers[6,8,9]; the present results emphasize their findings. Reference to Tables I and II will show that, according to CO_2-output and O_2-uptake data, tyrosine and aspartic acid were both oxidized to CO_2 to the extent of some 68–69% in the garden soil, both left a nitrogenous organic residue with C : N ratio = 3.3 in the soil. Yet only 22% of the nitrogen of tyrosine was mineralized compared with 68% of the nitrogen of aspartic acid. It therefore seems probable that, in any study of the breakdown of nitrogenous organic materials in soil, methods such as we have described, that measure the extent of decomposition of the quantitatively more important carbonaceous moiety of the organic material, will prove more useful than methods depending upon measurement of the mineralization of organic nitrogen.

SUMMARY

1. The kinetics of decomposition of 17 common amino acids in a garden soil have been followed by percolation techniques that permitted the measurement of O_2-uptake and CO_2-output consequent upon decomposition.

2. Most of the amino acids decomposed rapidly; methionine and threonine decomposed much more slowly.

3. After decomposition of amino acids in soil, the material residual in the soil had a C : N ratio of about 3 : 1; this possibly represents microbial protein.

4. The significance of the results, and the potentialities of the manometric techniques used, are discussed.

ACKNOWLEDGEMENTS

We wish to acknowledge our debt to Dr. W. T. H. Williamson and Professor W. O. Kermack, F.R.S., for their help, advice, and encouragement throughout this work. One of us (D.J.G.) would record his thanks to the Lancashire County Council for their generous provision of Financial Aid to enable his part in this work to be carried out.

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UNTERSUCHUNGEN MIT BODENVERBESSERUNGSMITTELN

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Aus der Erkenntnis heraus, daß gesicherte Höchsterträge auf die Dauer mit Mineraldüngern allein nicht erzielt werden können, wird der praktischen Landwirtschaft seit Jahren geraten, den Humuszustand des Bodens in Ordnung zu halten. Sorgfältige Gewinnung und Anwendung der wirtschaftseigenen organischen Dünger in einer humusmehrenden Fruchtfolge erhalten die Fruchtbarkeit des Bodens. In Gebieten aber, in denen die organischen Düngemittel nicht ausreichen, oder ein Abbau dieser so stürmisch ist, daß nicht genügend organische Substanz nachgeliefert werden kann, muß nach anderen Mitteln und Wegen zur Humuserhaltung bzw. zur Humusanreicherung gesucht werden. Für deutsche Verhältnisse sind Gründüngung und Zwischenfruchtbau bei ausreichenden Niederschlägen Wege zu dem gesteckten Ziel [22]. Aber nicht in allen Ländern ist eine organische Düngung möglich oder üblich und auch Zwischenfruchtbau nicht durchzuführen. In solchen Gebieten gibt es praktisch keine organische Substanz im Boden mehr. Es ist daher nicht weiter verwunderlich, daß keine Mühen gescheut werden, um auch bei solchen schwierigen Böden und Lagen Wege zu finden, den Boden zu verbessern.

Krilium, Kapokril, Flotal u.a. sind Ergebnisse dieser Bemühungen. Diese Bodenverbesserungsmittel sollen den Anteil der beständigen Krümel erhöhen, den Luft- und Wasserhaushalt des Bodens verbessern und so den Boden bei gleichbleibendem Mineraldüngeraufwand befähigen, höhere Erträge zu liefern.

Verschiedene Autoren [2 6 7 9 10 11 12 13 19 20 21 23 24] beschäftigen sich mit der Wirkung von Krilium und ähnlichen Präparaten auf die

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Bodenstruktur, mit der Wasserhaltekraft und der ertragssteigernden Wirkung. Übereinstimmend zeigen alle Autoren, daß die Präparate in Konzentrationen um 0,2% den Anteil der wasserbeständigen Krümel erhöhen [2 6 7 9 10 11 13 21 23 24]. Dagegen werden die Wasserhaltekräfte [2 7 10 13 19 20 23] und die ertragssteigernde Wirkung [7 10 12 13 24] unterschiedlich beurteilt. Die Untersuchungen von Czeratzki[3] und Flaig[4] zeigen, daß Krilium und ähnliche Präparate in der Lage sind, den Boden in einen krümelnden Zustand zu versetzen. Sie vertreten aber mit Recht die Ansicht, daß diese Präparate die breite Praxis in Deutschland vorerst noch nicht beschäftigen sollten.

Flotal, Krilium und Kapokril wurden von uns in ihrer Wirkung auf die Krümelstabilität, auf die Keimung von Roggen und auf die Löslichkeit von P_2O_5 und K_2O im Boden geprüft.

Die Präparate

1. Flotal ist ein Erzeugnis der italienischen Firma „Rumianca", Turin. Es ist ein Mischprodukt mit eisenhaltigen Salzen, die an einen organischen Träger von hoher Sorptionskraft gebunden sind. Flotal soll 80% Eisenammon-Alaun und 20% Torf enthalten [15]. Das Mischprodukt enthält 34% bei 105° flüchtige Bestandteile, 16% org. Substanz, 13,8% Asche, 2,6% SiO_2, 12,7% Fe_2O_3, 34,9% SO_4, 3,1% N sowie noch Alkalien und Erdalkalien.

2. Krilium (Formulation 9) ist das Natriumsalz (20% Na_2O) eines teilweise hydrolysierten Polyakrylnitrits und ähnelt der Orlon- oder PAN-Faser. Es ist ein Polyanion mit mehr als 100 negativen Ladungen je Molekül. (Molekulargewicht *ca.* 50 000) [8].

3. Krilium (Formulation 6) ist dieselbe Verbindung als Ca- oder K-Salz. Beide Kriliumarten werden von der Monsanto Chemical Company USA hergestellt.

4. Kapokril-T Präparat Nr. : 4453, ein deutsches Erzeugnis der Bayer-Werke Leverkusen, ist uns in seiner Zusammensetzung nicht bekannt (Na_2O-Gehalt 3%).

Krilium und Kapokril dürften ähnlich aufgebaut sein. Sie unterscheiden sich aber erheblich vom Flotal. Das Mischprodukt Flotal hat mit seinem hohen Gehalt an Fe_2O_3 für italienische Böden [16 17 18] eine ähnliche Bedeutung wie der Kalk für deutsche. Wie aus unseren Arbeiten zu schließen ist, haben Krilium und Kapokril den Charakter von Klebstoffen, die fein verteilt auf den Boden gebracht, diesen zu größeren Aggregaten (Krümel?) verbinden.

EXPERIMENTELLER TEIL

I. *Versuche mit Flotal*

In 14 Neubauerschalen wurden je 400 g lufttrockener Reinhäuser Ton eingewogen, der vorher auf 2 mm abgesiebt worden war. Zu den Gefäßen wurden folgende Mengen Flotal gegeben: 0–10–200–400–1000–2000–4000 mg. Der Boden enthielt jetzt 0–1% Flotal. Jede Dosierung wurde mit einer Parallele angesetzt.

a) Bestimmung der Wasserverdunstung flotalhaltigen Bodens. Zu den Böden wurden pro Neubauerschale 200 ccm Wasser gegeben und alle 2 Tage der Verlust an Verdunstungswasser durch Wägen ermittelt. Nach 17 Tagen waren im Durchschnitt etwa 130 ccm Wasser verdunstet. Es zeigte sich zwischen dem behandelten und unbehandelten Boden kein Unterschied. Der Boden wurde nun abermals auf seinen ursprünglichen Wassergehalt gebracht und nach weiteren 18 Tagen konnte die gleiche Feststellung gemacht werden. Boden aus dem Untergrund des Göttinger Versuchsfeldes zeigte ebenfalls keine Veränderung der Verdunstung, obwohl in diesem Versuch bis zu 4% Flotal zum Boden gegeben worden waren.

b) Bestimmung der Nährstoffestlegung durch Flotal. Der Boden wurde nun wieder zerkleinert, auf 2 mm abgesiebt und nach der Laktatmethode untersucht. Bei der Kalilöslichkeit zeigten sich keine Unterschiede, wohl aber bei der P_2O_5-Löslichkeit, die mit steigenden Gaben erheblich zurückging, wie Tabelle I zeigt. Auch das pH zeigt sinkende Tendenz. Der gleiche Versuch mit einem Boden aus dem Untergrund des Göttinger Versuchsfeldes zeigte am Ende der Versuchszeit bei 4% Flotal ein pH KCl von 3,4

TABELLE I

Ergebnis der Bodenuntersuchung flotalhaltiger Böden

Flotal % im Boden	pH/KCl	mg/100 g Boden	
		P_2O_5	K_2O
0	7,32	10,0	32
0,025	7,26	10,0	30
0,05	7,25	10,0	28
0,10	7,22	9,5	30
0,25	7,15	8,8	29
0,5	7,12	7,4	31
1,0	7,11	4,4	31

(Ausgangs-pH 7,2). Auch die P_2O_5- und K_2O-Löslichkeit nahmen ab. Das niedrige pH erklärt die weiter unten erwähnte Keimschädigung bei Roggen und die niedrigen P_2O_5-Werte. Im sauren Bereich wird P_2O_5 durch das Eisen des Flotals festgelegt. Die sinkende Tendenz der K_2O-Werte hat ihre Ursache im Torf des Flotals.

c) Bestimmung der krümelstabilisierenden Wirkung. Verschiedene Arbeiten über den Anteil beständiger Krümel im Boden hatten gezeigt, daß die bisher von uns verwandte Schlämmapparatur nach Kopecky-Krauss zwar gute Ergebnisse lieferte, in der Durchführung aber zu zeitraubend war. Wir haben daher eine Schnellmethode entwickelt, die mit der in der Abbildung 1 gezeigten Apparatur arbeitet.

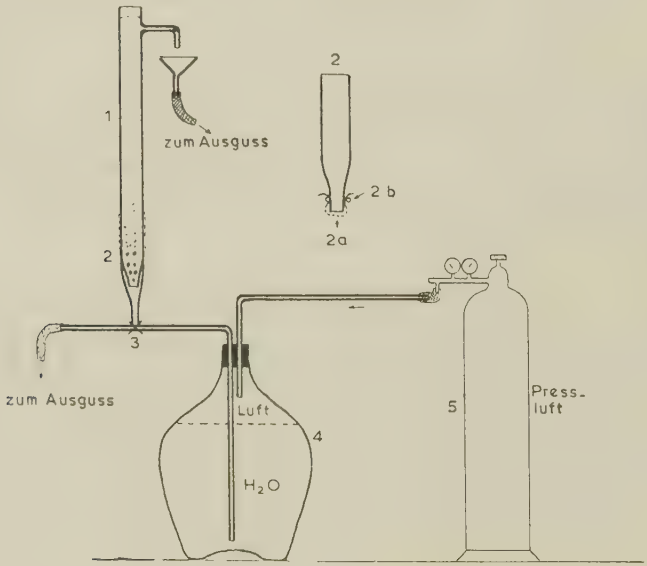

Abb. 1. Apparatur zur Bestimmung der beständigen Krümel.

1 = Spülrohr mit Überlauf,
2 = Konisches Einfüllrohr zur Aufnahme des Bodens,
2*a* = Gaze-Verschluß,
2*b* = Gummiring,
3 = T-Stück mit Dreiwegehahn,
4 = Ballon mit Wasser,
5 = Preßluftbombe.

In das konische, am dünnen Ende durch Gaze verschlossene Einfüllrohr (2) werden genau 10 g lufttrockener, auf 1–2 mm abgesiebter Boden eingewogen.

Dieses Einfüllrohr wird mittels eines Schlauches am unteren Ende des Spülrohrs (1) angeschlossen, wobei sich die beiden Glasrohre berühren müssen, damit die Spülhöhe immer konstant ist. Das dünne Ende des Einfüllrohrs wird nun an einen Ballon *Aqua dest.* angeschlossen, der wiederum mit einer Preßluftbombe so verbunden ist, daß die Preßluft das *Aqua dest.* in den Spülzylinder drückt. Der Miniskus im Spülrohr soll 5 cm über dem Auslauf des Spülrohrs stehen, damit die Spülhöhe und die auslaufende Wassermenge konstant bleiben. Dazu sind etwa 0,2 atü erforderlich. Der Spülvorgang dauert vom Auslaufen des ersten Tropfen Wassers ab genau 10 Minuten, wobei 7,5 Liter Wasser durch den Boden gespült werden. Nach 10 Minuten wird der Dreiwegehahn (3) so gedreht, daß das Wasser aus dem Vorratsgefäß zum Ausguß fließt, die Preßluft abgestellt und dann der Dreiwegehahn so gestellt, daß nun das Wasser im Spülrohr nach unten in den Ausguß abfließt. Der Boden setzt sich nun im Einfüllrohr ab. Dieses wird herausgenommen und der Boden mittels wenig Wasser in ein trockenes, gewogenes, 100 ccm fassendes Becherglas gespült. Der Boden wird nun bis zur Gewichtskonstanz im Trockenschrank getrocknet. Parallel dazu wird vom eingefüllten Boden eine Trockensubstanzbestimmung gemacht und das Trockengewicht des im Einfüllrohr gebliebenen Bodens darauf bezogen. Diese Apparatur erlaubt es, in einer kurzen Zeit eine große Anzahl von Böden zu untersuchen.

Vergleichende Untersuchungen mit dieser Apparatur und mit der Schlämmapparatur lieferten gleiche Ergebnisse. Den Einfluß des Flotals auf den Anteil der beständigen Krümel in der oben beschriebenen Apparatur zeigt Tabelle II.

TABELLE II *

Der Einfluß von Flotal auf die Krümelbeständigkeit					
Fotal % im Boden	M g	m +—	m% +—	Beständige Krümel in %	Unbehandelt = 100
0	2,895	0,216	7,5	29,0	100
0,025	2,570	0,209	8,1	25,7	89
0,05	2,548	0,185	7,2	25,5	88
0,1	2,734	0,220	12,7	27,3	94
0,25	3,038	0,192	6,3	30,4	105
0,50	2,892	0,096	3,3	28,9	100
1,0	3,078	0,091	3,0	30,8	106

* Berechnungen von „m" USW. in den Tabellen II, IV und V nach Mudra[25].

Die Ergebnisse zeigen, daß durch Flotal der Anteil der wasserbeständigen Krümel nicht erhöht wird. In einer früheren Versuchsreihe mit einem anderen Boden wurde bei mehr als 2% Flotal ein Einfluß insofern beobachtet, als der Boden trotz gleichen Wassergehalts ein trockenes Aussehen hatte. Mit steigender Flotalgabe ab 1% war der Boden locker gelagert und fühlte sich krümelig an, während der

Kontrollboden und der Boden mit niedrigen Flotalgaben nach dem Eintrocknen sich stark verfestigten. Die Krümel konnten aber nach beiden genannten Untersuchungsmethoden nicht als wasserstabil bezeichnet werden.

d) Der Einfluß von Flotal auf die Entwicklung von Roggenkeimlingen

100 g des mit Flotal gemischten Bodens wurden in Neubauerschalen mit 100 g Quarzsand gemischt und mit weiteren 100 g Quarzsand abgedeckt. Dann wurden 50 ccm *Aqua dest.* zugegeben, 100 Roggenkörner (Gesamtgewicht 3,8 g) eingelegt, diese wieder mit 100 g Quarzsand abgedeckt und mit weiteren 30 ccm *Aqua dest.* angefeuchtet. Die so vorbereiteten Schalen wurden gewogen und 17 Tage im Warmhaus bei 15–20°C gehalten. Nach Auflaufen der Keimlinge wurde die verdunstete Wassermenge täglich ersetzt.

Während der Wachstumszeit zeigte sich kein Unterschied in der Entwicklung der Keimlinge auf den unterschiedlich behandelten Böden. Nach 17 Tagen wurden die Halme und die sauber gereinigten Wurzeln getrennt geerntet und der Trockensubstanzgehalt ermittelt. In der Trockensubstanz wurde P_2O_5 kolorimetrisch nach der Molybdän-Vanadat-Methode und K_2O sowie Na_2O flammenphotometrisch bestimmt. Die Werte gibt die Tabelle III wieder.

TABELLE III

Flotal % im Boden	Trockengewicht g	Analysenergebnisse ± mg	% P_2O_5	% K_2O	% Na_2O
Halme					
0	1,127	19	2,04	4,44	4,4
0,025	1,142	11	2,24	5,86	4,2
0,05	1,195	3	2,22	5,99	4,0
0,10	1,151	27	2,26	6,20	4,2
0,25	1,156	51	2,25	6,28	4,2
0,50	1,222	37	2,20	4,39	3,5
1,0	1,241	69	2,01	6,76	3,9
Wurzeln					
0	0,982	35	0,87	1,02	2,2
0,025	0,944	2	0,93	1,27	2,1
0,05	0,931	9	0,94	1,18	2,5
0,10	0,963	5	0,88	1,14	1,7
0,25	0,932	22	0,83	1,02	1,8
0,50	0,870	2	0,81	1,27	2,6
1,0	0,876	36	0,88	0,91	2,2

Nach obigen Analysen werden das Trockengewicht der Wurzeln, der P_2O_5-Gehalt und der Na_2O-Gehalt in Halmen und Wurzeln nicht verändert. Das Trockengewicht der Halme und ihr K_2O-Gehalt steigen etwas an, was vielleicht auf einen N- bzw. K_2O-Gehalt des Flotals zurückgeführt werden kann. In einer anderen Versuchsreihe mit Flotalgaben bis zu 4% traten im Neubauerversuch starke Keimschäden auf.

Interessant ist, daß die nach der Laktat-Methode gefundene Abnahme der P_2O_5-Löslichkeit mit steigender Flotalgabe im Neubauer-Versuch nicht deutlich erkennbar ist. Durch den hohen Fe-Gehalt des Flotals werden wahrscheinlich Eisenphosphate gebildet, die nicht laktatlöslich, wohl aber pflanzenaufnehmbar sind.

II. *Versuche mit Krilium * und Kapokril.*

14 Neubauerschalen erhielten je 400 g lufttrockenen Reinhäuser Ton, der vorher auf 2 mm abgesiebt worden war. In 12 der Gefäße wurden steigende Mengen Krilium gegeben, und zwar 100–200–300–400–600–800 mg Krilium. Jede Dosierung wurde mit einer Parallele angesetzt. Zwei Gefäße blieben als Kontrolle unbehandelt. Der Boden enthielt jetzt 0–0,025–0,05–0,075–0,10–0,15–0,20% Krilium.

a) Bestimmung der Wasserverdunstung kriliumhaltigen Bodens. (Methodik wie unter I*a* beschrieben). Nach einer Inkubationszeit von 35 Tagen zeigte sich, daß *durch Krilium im Boden die Wasserverdunstung nicht verändert wurde* (siehe dazu [2 7 10 13 19 20]. Nach weiteren 20 Tagen war der Boden hart eingetrocknet und bildete Risse. Diese Risse waren bei den höheren Kriliumgaben an mehreren Stellen durch farblose, zähe Bänder von etwa 0,1 mm Ø verbunden. *Kriliumfäden überspannten also die Risse und Spalten des Bodens.*

b) Bestimmung der Nährstoffestlegung durch Krilium. Der Boden wurde nun wieder zerkleinert und auf 2 mm abgesiebt. Die Untersuchung nach der Laktat-Methode auf P_2O_5 und K_2O brachte keinen Unterschied, der durch Krilium verursacht worden sein konnte. Auch das pH wurde nicht verändert. Bei der flammenphotometrischen Bestimmung des K_2O zeigte die Acetylenflamme mit steigender Kriliumgabe eine stärkere Gelbfärbung, die auf den hohen Na-Gehalt des Kriliums (Formulation 9) zurückzuführen ist.

c) Bestimmung der krümelstabilisierenden

* Bei allen Versuchen mit Krilium wurde „Formulation 9" verwandt, da von der „Formulation 6" nur eine kleine Probe vorhanden war.

Wirkung des Kriliums. Der Anteil der wasserbeständigen Krümel wurde nach der oben beschriebenen Methode ermittelt. Die Ergebnisse siehe Tabelle IV.

TABLELE IV

Mittelwerte und Anteile der beständigen Krümel in Prozent							
Krilium-gehalt %	M g	m ±	m% ±	S.W.	Bemerkung zur Sicherung der Differenz	Beständige Krümel in % der Tr. Subst.	Unbehand. = 100
0	3,02	0,13	4,3	—	—	30,9	100
0,025	3,21	0,17	5,3	0	nicht ges.	32,8	106
0,05	3,44	0,03	0,9	1	sehr gut ges.	35,2	114
0,075	4,08	0,17	4,2	1	,, ,, ,,	41,8	135
0,10	3,58	0,13	3,4	0,75	gut ges.	36,7	120
0,15	4,71	0,27	5,7	1	sehr gut ges.	48,4	156
0,20	5,42	0,08	1,5	1	,, ,, ,,	55,6	180

Nach unserer Untersuchungsmethode wurde gefunden, daß der Boden mit einem Gehalt von 0,2% Krilium 80% mehr stabile Krümel enthält als der unbehandelte Boden.

Der obige Versuch wurde in der gleichen Weise mit Krilium wiederholt und vergleichsweise dazu eine Reihe mit steigenden Gaben Kapokril angesetzt. Nach einer Inkubationszeit der Mittel von 4 Monaten, während der die Schalen mehrfach angefeuchtet wurden, wurde in der oben beschriebenen Apparatur die Krümelbeständigkeit ermittelt.

TABELLE V

Einfluß von Krilium und Kapokril auf die Krümelbeständigkeit						
Präparat mg	% Präparat im Bd.	M g	± m	± m %	Beständige Krümel i.d. lufttr. Subst. %	Unbehandelt = 100
Ohne	—	2,713	0,066	2,4	27,1	100
Krilium						
100	0,025	2,812	0,082	2.9	28,1	104
200	0,050	3,441	0,058	1,7	34,4	127
300	0,075	3,933	0,095	2,4	39,3	145
400	0,10	4,105	0,064	1,6	41,1	152
600	0,15	4,844	0,044	0,9	48,4	178
800	0,20	5,531	0,063	1,1	55,3	210
Kapokril						
100	0,025	3,130	0,040	1,3	31,4	116
200	0,050	3,918	0,138	3,5	39,2	145
300	0,075	3,583	0,129	3,6	35,8	132
400	0,10	3,946	0,099	2,5	39,5	146
600	0,15	4,405	0,099	2,2	44,1	163
800	0,20	4,893	0,037	0,8	48,9	180

Die Ergebnisse sind in der Tabelle V zusammengestellt. Sie lassen erkennen, daß der Anteil der im Wasserstrom beständigen Krümel gleichmäßig steigt. Er liegt bei der höchsten Gabe bei Krilium 110% und bei Kapokril 80% über dem des unbehandelten Bodens. Nach dem Ergebnis scheint Krilium ein wenig günstiger zu wirken. Es fällt auf, daß bei Kapokril die Schwankungen innerhalb der Parallelen etwas höher liegen als bei Krilium. Die Ursache dürfte in der gröberen Beschaffenheit des Kapokril gegenüber Krilium zu suchen sein, wodurch eine gleichmäßige Verteilung nicht so gut gewährleistet ist wie beim Krilium.

d) Beobachtung des Einflusses von Krilium und Kapokril auf die Entwicklung von Roggenkeimlingen im Neubauerversuch.

Mit 50 g — einem Rest des im ersten Versuch mit Krilium behandelten Bodens — wurde, wie bei Flotal beschrieben, ein Keimversuch angesetzt. Trockensubstanz- und Nährstoffmengen gibt Tabelle VI wieder.

TABELLE VI

Der Einfluß von Krilium auf die Keimlingsentwicklung von Roggen. Ergebnis der Analysen

Krilium-gehalt %	Trocken-subst. g	± mg	P_2O_5 %	K_2O %	Na_2O %
Halme					
0	1,135	2	1,96	5,28	5,4
0,025	1,133	9	1,89	4,76	6,9
0,05	1,112	7	1,77	4,49	7,8
0,075	1,066	0	1,93	4,58	7,2
0,10	1,105	23	1,95	4,70	8,2
0,15	1,137	2	2,00	4,32	8,4
0,20	1,115	23	1,96	4,75	8,0
Wurzeln					
0	0,896	15	0,75	1,23	1,7
0,025	1,022	49	0,84	1,22	1,5
0,05	0,983	23	0,82	1,40	1,9
0,075	0,917	3	0,87	1.20	2,1
0,10	0,884	32	0,88	1,13	2,9
0,15	0,882	9	0,82	0,90	3,9
0,20	0,845	21	0,77	0,71	3,9

Die Bildung an Trockensubstanz wird durch Krilium im Keimversuch mit Roggen während 17 Tagen bei Halm und Wurzel nicht verändert. Auch der P_2O_5-Gehalt bleibt gleich. Der Gehalt an Na_2O

dagegen steigt in Halmen und Wurzeln erheblich an, wodurch der K_2O-Gehalt etwas gedrückt wird. Dieses ist nicht weiter verwunderlich, da die Aufnahme von K_2O immer dann zurückgeht, wenn der Pflanze hohe Na_2O-Mengen geboten werden.

Ein weiterer Versuch, bei dem die in Tabelle VII angegebenen Mengen an Kapokril und Krilium zu sterilem Sand in Neubauerschalen gegeben wurden, zeigte ebenfalls keinen Einfluß dieser Präparate auf die Keimlingsentwicklung von Roggen. Die P_2O_5-Analysen liegen sämtlich im Schwankungsbereich der Untersuchungsmethode. Bei Krilium ähneln die K_2O-Analysen der Kontrolle, zeigen aber bei Kapokril eine starke steigende Tendenz mit steigender Gabe. Der Na-Gehalt steigt bei Krilium und Kapokril anfänglich an, hält sich dann aber auf gleicher Höhe. Bei Kapokril liegen die Na_2O-Gehalte niedriger als bei Krilium.

TABELLE VII

Der Einfluß von Krilium und Kapokril auf die Keimlingsentwicklung von Roggen

mg Präparat pro Schale	Trockensubstanz g	P_2O_5 %	K_2O %	Na_2O %
Wurzeln				
ohne	0,990	1,0	0,5	0,1
200 Krilium	1,005	0,9	0,5	0,9
400 „	1,007	0,8	0,4	0,7
600 „	0,853	1,0	0,5	0,8
200 Kapokril	1,015	0,9	0,9	0,4
400 „	1,061	0,9	1,2	0,4
600 „	1,165	0,8	1,5	0,4
Halme				
ohne	1,035	1,5	1,5	0,1
200 Krilium	1,203	1,5	1,7	0,5
400 „	1,063	1,4	1,7	0,5
600 „	1,311	1,4	1,3	0,6
200 Kapokril	1,172	1,5	3,4	0,2
400 „	1,272	1,6	4,3	0,2
600 „	1,367	1,5	5,2	0,2

ZUSAMMENFASSUNG

1. Flotal erhöhte den Anteil der wasserbeständigen Krümel nicht. Bis zu 150 dz Flotal pro ha wirkten sich auf den Versuchsboden nicht schädlich aus, höhere Gaben versauerten ihn und führten zur merklichen Festlegung von P_2O_5 und K_2O.

2. Durch Krilium werden die Wasserverdunstung des Bodens nicht verändert und die Löslichkeitsverhältnisse von Phosphorsäure und Kali sowie das pH nicht beeinflußt. Der Anteil der beständigen Krümel steigt mit der Kriliumgabe. Die Nährstoffaufnahme von Roggenkeimlingen bleibt bei P_2O_5 konstant, während die von Na_2O steigt, was auf den hohen Gehalt des Kriliums (Formulation 9) an Natrium zurückzuführen ist. Demzufolge sinkt die K_2O-Aufnahme.

3. Kapokril kommt in seiner krümelstabilisierenden Wirkung dem Krilium etwa gleich. Auf die Trockensubstanzbildung und den P_2O_5-Gehalt bei Roggenkeimlingen im Neubauerversuch ist Kapokril ohne Einfluß. Der Na_2O-Gehalt wird etwas -, der K_2O-Gehalt stark erhöht.

4. Eine Schnellmethode zur Bestimmung wasserstabiler Krümel wird beschrieben.

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PRODUCTION AND PREVENTION OF FRENCHING OF TOBACCO GROWN IN THE GREENHOUSE

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In spite of occasional reports to the contrary, no methods are known for the *de novo* production and the prevention of frenching in the tobacco plant. Inability to induce or maintain this physiological disease has prevented its systematic study. Its occurrence in nature is usually sporadic and often of brief duration. This situation is not improved by avoidance by the farmer of fields where frenching has occurred. These characteristics of the abnormality have led in the past to results often incapable of repetition.

Previous studies *in vitro* and field [6 7] had indicated that the cause of this condition was probably a common soil saprophyte, *Bacillus cereus*, Frankland and Frankland, whose diffusates acted as a soil toxin on the tobacco plant. Contact between plant and microorganism was unnecessary, and even the dead bacteria (sterilized old cultures) caused the appearance of severe frenching symptoms in healthy seedlings. L-soleucine, and especially L-alloisoleucine, caused tobacco in aseptic culture to french [7].

A limited field count revealed that a definite rise in soil and rhizosphere numbers of *B. cereus* accompanied frenching. The acidity of the soil was also concerned. Although frenching occurred over the full range of pH suitable for the plant, it was more infrequent at high acidities (pH 4.6- 5.2). The severity of the symptoms tended to be greater at low soil acidity, as did retentivity of frenching capacity of soil when brought into the greenhouse. Resumption of normal growth in side-shoots of affected plants was found not to be indicative of loss of frenching capacity in soils.

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Unpublished *in vitro* tests with soils in which frenching had occurred, moreover, had indicated that, with several exceptions, none of the fertilizer salts apparently affected the course of the abnormality to any consistent or great degree. The exceptions were sodium nitrate, lime, and dicalcium phosphate. The first prevented appearance of symptoms in the plant temporarily, the latter two resulted in their intensification. Cellulose also seemed to be of some aid in bringing about appearance of frenching symptoms [4], possibly because of decreases in available soil nitrogen through enhanced growth of micro-organisms. Heat, carbon tetrachloride and chloroform sterilization of "infectious" soils resulted in production of normal seedlings.

The method evolved for production of frenching capacity in sterilized normal soils was based on a combination of the above results. Interestingly enough, liming [3], among other factors, had already been suggested as the primary cause of frenching, as had low nitrogen [5 9]. The use of high soil temperature [2], although not found to be essential, also proved quite convenient as a time saver in this study. Use of a method of successive crops, however, was indispensable if results were to be obtained of a degree of uniformity capable of repetition. Failure to use this procedure may have been an important factor in the erratic results reported in the literature.

Miscellaneous experiments carried out with soil antiseptics and with manure under greenhouse conditions are not reported in this manuscript. The results, at least on the limited scale employed, were erratic and often contradictory. It was possible, however, to verify the report [1] that leaching of "infected" soils intensified symptoms of frenching in tobacco, whereas similar treatment of normal soils did not lead to production of frenching capacity.

EXPERIMENTAL METHODS

Yellow Mammoth tobacco, a flue-cured variety, was sowed in flats and the seedlings were later transplanted to two-inch pots. The seedlings, when of suitable size, were then transplanted to asphalted 7-inch pots after washing the roots free of soil. These were plunged into sand beds maintained at about 35°C. The length of time required for symptoms of frenching to appear and its degree was noted. When additional cases of frenching appeared only after long intervals, the

normal plants were decapitated and allowed to grow for several weeks longer. Duration of growth of a "crop" in the bed was usually between 2 to 3 months.

Neutral (pH = 7) soil for use in the induction experiments was prepared by mixing sterilized greenhouse soil with the following materials in the quantities indicated. Greenhouse sterilization was never complete.

Soil	41 potfuls (7″)
Sand	5 ,,
Hydrated lime	240 grams
Dicalcium phosphate	480 ,,
Cellulose (purified wood fiber)	480 ,,

A batch of this size was sufficient for filling 48 seven-inch pots, the number used in each hot-bed of about 17 × 5 feet in size. The procedure used was to fill the pots with this soil mixture, add any special ingredient to each individual pot and then the contents of each pot was mixed separately. The pots of soil were kept moist, while on a greenhouse bench without bottom heat, for several months before use in the hot-beds. Four pots were used for each treatment, their positions in the bed being randomized in each of 4 blocks. Successive plantings("crops") were made in these pots without further treatment until responses were general.

The soil in the pots used for the induction experiments was used again after being mixed as a single lot in the experiments on prevention of frenching. Three levels of acidity and four of potassium nitrate supply were used in these tests. Procedures were the same otherwise as in the induction tests. The acidity levels were produced by addition of powdered sulfur to the soil.

The soils successively used in these experiments were supplied for general greenhouse use and were obtained from three different sources. The first was from a truck farm section in northeastern Virginia, the second from an uncultivated field adjacent to the northern limit of tobacco farming in Maryland, and the last from an Agricultural Research Center building project. None of these soils, with the possible exception of the second, had ever been previously used for cultivation of tobacco as far as is known. None of these soils when used for ordinary potting purposes in the greenhouse produced frenching in tobacco.

Symptoms of frenching in tobacco varied depending on severity. The earliest and mildest stage consisted of a network or reticular chlorosis of the younger leaves. This stage might be transient, or might be followed by failure of the leaves to expand fully in width.

In the latter case, successively developed leaves might be formed with a progressive decrease in leaf width (strap-leaves). Inhibition of stem growth and formation of flower buds often accompanied these symptoms; or bud necrosis took place later. Severely affected plants grown for very long periods have been observed to form normal branches, even though the frenching capacity of the soil had not decreased. Strap-leaf formation was observed in more than 90 per cent of the plants that frenched.

Symptoms were subject to some modification through manipulation. The reticular chlorosis stage could often be by-passed by growth with very low soil nitrogen or transplanting after washing of roots. Use of graduated quantities of nitrogen or fertilizer (4–8–12) or transplanting seedlings without disturbing the roots delayed appearance of frenching symptoms, or even prevented the appearance of strap-leaves.

None of the preliminary studies carried out during 1949 to 1952 in working out the conditions required for induction and prevention of frenching have been tabulated in this paper. The data submitted are considered to afford ample proof of ability to induce and prevent frenching of tobacco in the greenhouse.

FRENCHING OF TOBACCO IN SUCCESSIVE CROPS

The values assembled in Table I demonstrate the cumulative effect of successive plantings on the frenching capacity of the soil mixture employed. The soil additions indicated in column I were made prior to the first crop and were not repeated with succeeding crops. There were four crops in all. It will be noted that both percentage and rapidity of frenching increased progressively with each succeeding crop as a rule. This occurred both with the control plants and those growing in soil to which various additions had been made. Attention is called to the fact that additional quantities of cellulose over and above those present in the initial soil mixture did not greatly affect the progress of frenching. Additional dicalcium phosphate did, however, appear to increase rapidity of response appreciably.

The plants in the soil treatments selected for reproduction in this table showed similar variations in frenching responses to the succes sive crop procedure. The final results in the fourth crops were much the same no matter what initial addition to the soil had been

made. However, uramon (urea) did appear to display a definite delaying action on the development of frenching symptoms. All trials illustrated the ability of soils to increase in frenching capacity through continuous planting of tobacco when an adequate level of fertilization was not maintained.

TABLE I

Frenching of successive crops of Yellow Mammoth tobacco after a single initial addition to a sterilized soil mixture

Soil additive *		Crop							
Compound	Per 7″ pot	1		2		3		4	
	g	%	Days	%	Days	%	Days	%	Days
No addition . .	—	0	—	31	34	81	24	88	21
$CaHPO_4.2H_2O$.	20	0	—	50	40	100	14	100	12
Cellulose	10	0	—	25	30	100	23		
Uramon	5	0	—	25	38	100	28	75	28
4–8–12 fertilizer .	5	25	43	50	36	100	14		
Average		5	43	36	36	96	21	88	20

* 5g/7″ pot is equivalent to 3100 pounds per acre (on basis of weight of soil per pot compared to a 2,000,000 pound acre).

Similar responses to successive plantings took place with initial addition of other fertilizer ingredients. These data are arranged in Table II according to fertilizer salt level and crop number. In almost every case each addition of a salt to soil resulted in a similar response: a steady increase in frenching capacity of the soil with successive plantings, exactly as in the controls. Uusally none of the plants of the first crop frenched. Both the 2- and 5-g salt levels led to similar responses.

There was some evidence for the existence of two intermediate specific effects of salt addition. One of these was a delaying action of nitrogen salts on the development of frenching in the second planting. The other consisted of an intensification of the frenching effect by higher phosphate. These responses were obtained at both levels of addition to the soil.

The delaying action of nitrogen fertilization on development of symptoms of frenching was checked at three levels of soil acidity (Table III). Potassium nitrate was used as a source of nitrogen and sulfur as a source of soil acidity. Each value is the average of three

TABLE II

Effect of fertilizer salts on percentage and rapidity of frenching in successive tobacco plantings *

Cation	Anions of salts at 2 g/7″ pot level						Anions of salts at 5 g/7″ pot level					
	NO_3		SO_4		PO_4		NO_3		SO_4		PO_4	
	%	Days	%	Days	%	Days	%	Days	%	Days	%	Days
First planting												
NH_4	0	—	0	—	25	51	0	—	0	—	0	—
K	0	—	0	—	0	—	0	—	0	—	0	—
Mg	0	—	0	—	25	46	0	—	0	—	25	42
Ca	0	—	0	—			0	—	75	33		
Second planting												
NH_4	0	—	100	35	25	16	0	—	0	—	0	—
K	100	40	75	38	0	—	0	—	25	32	25	35
Mg	50	38	50	37	50	29	50	36	25	34	50	27
Ca	50	45	0	—			0	—	100	33		
Third planting												
NH_4	100	23	100	21	75	21	50	16	100	39	75	14
K	100	24	100	36	50	15	100	37	63	24	25	17
Mg	100	26	50	35	100	21	100	31	63	23	100	16
Ca	100	29	100	34			100	46	100	32		
Fourth planting												
NH_4					75	36			75	39	75	21
K			100	24	100	33			100	28	75	25
Mg			75	30					75	22		
Ca									100	30		

* Controls: first crop — 0%, — days; second crop — 63%, 34 days; third crop — 81%, 24 days; fourth crop — 88%, 21 days.

tests for a total of twelve plants, or a grand total of 576 plants grown over a period of several years.

Diminution of frenching capacity of soil took place both with increased nitrate and increased acidity. These effects were more evident with respect to the number of plants that frenched and less evident as concerns number of days required for frenching. Intensity of frenching seemed to parallel these quantitative data in that low acidity and nitrogen led most frequently to extreme frenching (strap-leaf formation).

Average values for percentage frenching decreased at each level of acidity with increase in potassium nitrate, whereas number of days for frenching decreased. At pH 6.98 a total of 64% frenched in 27 days; at pH 5.13, 20% in 22 days and at pH 4.40, 9% in 24 days. Averaging for potassium nitrate levels irrespective of acidity, the percentage frenching and the corresponding number of days were:

TABLE III

Effect of acidity and potassium nitrate levels on frenching of successive plantings of Yellow Mammoth tobacco.

Treatments *		Crop									
Acidity	KNO_3, g per 7″ pot	1		2		3		4		Average	
		%	Days	%	Days	%	Days	%	Days	%	Days
6.98	0	100	23	100	18	100	18	67	20	92	20
	1.4	92	28	83	21	50	35	67	27	73	28
	2.8	75	30	50	19	33	36	33	33	48	30
	4.2	67	25	42	15	33	46	33	33	44	30
5.13	0	67	18	0	—	33	19	0	—	25	19
	1.4	67	18	0	—	17	26	0	—	21	22
	2.8	33	21	0	—	33	24	0	—	17	23
	4.2	50	22	0	—	17	28	0	—	17	25
4.40	0	33	19	0	—	8	27	0	—	10	23
	1.4	33	21	0	—	8	19	0	—	10	20
	2.8	25	24	0	—	0	—	8	—	8	26
	4.2	25	24	0	—	0	—	8	—	8	26

* Potassium nitrate at 1.4 g per 7-inch pot is equivalent to 872 pounds per acre; it was added prior to each planting (on basis of weight of soil per pot compared to a 2,000,000 pound acre).

control —42%, 21 days; 1.4 g/pot —35%, 23 days; 2.8 g/pot —24%, 26 days; and 4.2 g/pot —23% and 27 days.

Overall frenching totals in one series of experiments with the last of the three soils were as follows. A total of twelve successive plantings (48 plants each) were made in the same batch of soil. Succeeding crops, including controls and fertilizer tests, gave 8, 29, 50, 77 and 92 per cent frenching. Overall frenching in the subsequent prevention experiments averaged 17, 13, 13 and 17 per cent. In later plantings discontinuance of potassium nitrate additions led to 29, 54 and 54 per cent frenching in successive crops.

DISCUSSION

These data were in agreement with the results of the writer's earlier unpublished experiment *in vitro*. Frenching was associated with low soil acidity, low nitrogen and high phosphate; and could be produced by continuous culture in a partially sterilized normal soil through addition of appropriate quantities of lime, dicalcium

phosphate and cellulose. Soil capacity for frenching in tobacco built up with successive plantings in the greenhouse to give severe leaf strapping in fourteen days or less. Transplanting seedlings with a ball of earth delayed appearance of frenching and frequently caused appearance of milder symptoms (reticular or network chlorosis of leaves only). Use of lower soil temperature had a smiilar delaying effect.

The converse of these results was also demonstrated in the "cure" experiments with sulfur and potassium nitrate. Increased acidity and nitrate delayed and diminished the symptoms of frenching. These results were not a consequence of "cure" but only of alleviation. Cessation of addition of potassium nitrate to each crop led to a resumption of frenching in soils to which normal seedlings were transplanted.

Counts of *Bacillus cereus* Fr. and Fr. in the soil and rhizospheres were made through the kindness of Dr. Francis E. Clark. Counts of *B. cereus* were found to be exceptionally high even with unfrenched plants though the total bacterial count remained unchanged. It would seem, therefore, that the soil amendments employed to produce frenching in tobacco also led to selectively high populations of *B. cereus*. This result coincides with the assumption that this micro-organism releases an organic toxin into the soil and that this toxin is the primary cause of frenching in tobacco [7]. The cause for occasional "escapes" is unknown but could be due to the sporadic presence of ineffective strains of *B. cereus*; or occasional high counts of other micro-organisms capable of rapidly destroying the soil toxin as formed.

It is of interest to compare the observational and experimental facts about the physiological disease known as frenching with the assumption that an organic toxin formed by *B. cereus* is the primary cause. Frenching may be prevented by soil sterilization with heat and formaldehyde [4], chloroform and carbon tetrachloride. High soil temperature aids production of frenching [2], perhaps through selectively increased growth of *B. cereus*. Diffusion products from this micro-organism can produce symptoms of extreme frenching in tobacco *in vitro* under aseptic conditions. Addition of either L-isoleucine (20 ppm) or L-alloisoleucine (4 ppm) are highly effective. Both are known to be formed by bacteria. Leaves of frenched plants are high in L-isoleucine [8]. Soil and rhizosphere counts of *B. cereus*

are appreciably higher when frenching of tobacco takes place in the field. Induction of frenching capacity in partially sterilized greenhouse soils also led to selectively high populations of *B. cereus*.

SUMMARY

The induction and elimination of frenching capacity in soils was studied in the greenhouse. It was found possible to induce this physiological disease in tobacco plants grown in normal soils following partial steam sterilization. High counts of *Bacillus cereus* Frankland and Frankland, the assumed causal agent, also resulted. Lime was added to soil neutrality, together with high dicalcium phosphate and a moderate quantity of cellulose. Growing plants with bottom heat (35 C) was of great aid in shortening duration of tests. Use of a method of successive plantings progressively depleted the soil of nitrogen and was necessary to obtain uniform and reproduceable results. Results with fertilizer ingredients were inconclusive except that low soil nitrogen, low acidity and high phosphate tended to increase frenching. Nitrogen deficiency and frenching symptoms were distinctly different and appeared independently of each other.

The converse experiments at three levels of soil acidity and four of potassium nitrate gave coordinating results. Acidity and abundant nitrate diminished or prevented frenching of the plants, without however eliminating frenching capacity of the soil.

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THE EFFECT OF LEVELS OF CALCIUM SULPHATE ON THE YIELD AND COMPOSITION OF A GRASS AND CLOVER PASTURE

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INTRODUCTION

Sulphur has recently been shown to be of some considerable significance in pasture production in Australia and New Zealand and the subject has been reviewed by Walker [5]. Legumes in the pasture appear to respond primarily [2 7], subsequent increased yields of grasses depending on underground transference of N or the return of N in animal excreta after grazing. In New Zealand clovers are relied upon almost exclusively as the source of the large amounts of N concerned in high pasture production [6 9], and until recently the phosphate in superphosphate (almost the only fertiliser used in New Zealand) has been credited with the greatly increased production which normally follows its application. This experiment was designed to assess the effect of sulphate applied as gypsum on the growth and composition of clover and grass in an ungrazed sward.

EXPERIMENTAL

The trial area which had received little if any manurial treatment was a 30-year old sown pasture on what was originally natural tussock grassland. The cover was mainly sweet vernal (*Anthoxanthum odoratum*), browntop (*Agrostis tenuis*), cocksfoot (*Dactylis glomerata*), very sparse and stunted white clover (*Trifolium repens*), cats-ear (*Hypochaeris radicata*), and occasional clumps of poa tussock (*Poa caespitosa*).

Soil

The soil type was Kowai sandy loam, a young loessial soil with about 8 inches of brown-grey sandy loam of weak structure on yellowish brown gritty

subsoil, situated on a high river terrace at 1400 ft in the Canterbury foothills of the Southern Alps. The analysis of the 0–4″ layer was as follows:

pH	T.E.C. me %	Exch. Ca me %	Exch. Mg me %	Exch. K me %	% Base saturation	Exch. Mn ppm	Easily reducible Mn ppm
5.9	5.6	4.4	1.9	1.0	84.9	5.1	33

Truog P_2O_5 mg %	% C	% N	% S	C/N	C/S	N/S
34	2.0	0.14	0.019	14	105	7.4

Treatments

The whole area received a basal dressing of triple superphosphate at 100 lb per acre, although the "available" phosphate content of the soil was high. Plot size was 5 sq.yds. with 4 replicates of the following treatments in randomised blocks: 0, 25, 50, 100, 200 lb gypsum per acre. The area was oversown with a mixture of legumes (N.Z. certified white clover, subterranean clover, Montgomery red clover and alsike clover) on 14 th October 1954, fenced off from stock and harvested on 21st April 1955 by cutting 1 sq.yd. from each plot with hand shears. Clovers were separated from grass and weeds and the two components dried and weighed for analysis. Soil samples (0″–4″ and 4″–8″) were also taken at the same time from replicates and bulked for the determination of residual sulphate.

Rainfall

The rainfall at the experimental site during the period of the trial was as follows:

	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	Total
Rainfall (inches)	1.20	1.24	6.72	2.03	5.33	2.00	0.28	18.80
Free surface evaporation (inches)	2.28	5.93	4.62	7.91	5.24	3.77	2.13	31.88

The rainfall was rather less than average (yearly average 45″) and two-thirds of the rain fell in the two months December and February. In this area evaporation is usually intense due to long hours of sunshine and strong Föhn winds. For comparison the data for evaporation from a free surface recorded at Lincoln (some 50 miles away) are presented; they are probably less than at the experimental site. It is quite clear that shortage of water restricted growth to at least a moderate degree in some months, particularly as the moisture-holding capacity of the soil was rather low on account of its coarse texture.

RESULTS

Yield

Yields of dry matter are presented in Table I. Whilst the experiment as a whole was subject to rather a high standard error per plot,

TABLE I

Mean yields of dry matter lb per acre							
Crop	Rates of gypsum					Difference for significance at 5% level	Standard error per plot %
	0	25	50	100	200		
Clover	40	196	608	674	708	148	23.6
Grass	813	920	1231	1412	1594	349	20.7
Total	853	1116	1839	2086	2302	454	19.6

differences between treatments were in most cases large and significant increases in yield were noted for both clover and grass. There was almost an 18-fold increase in yield of clover and the grass yield was about doubled, giving almost a three-fold increase in total production. The highest yield was still rather low but this could be expected to increase in subsequent years. In a previous experiment in a nearby field clover growth was increased from 67 to 866 lb dry matter per acre by a dressing of 100 lb gypsum per acre [7]. A point not brought out in the table was the much bigger contribution from cocksfoot at the higher levels of application of gypsum.

Yields of N are shown in Fig. 1. They follow the same pattern as the dry matter responses, clover N increasing from 1.1 to 24.4, grass N from 10.9 to 27.6 and the total yield from 12 to 52 lb per acre. This represents a four-fold increase in yield of N compared with the three-fold increase in yield of dry matter. A typical S-shaped response curve was obtained for clover N. The major part of the response came from the 50 lb dressing of gypsum, but a further slight response came from the higher dressings. The total yield of N was significantly higher for the 200 than for the 50 lb rate.

Composition of clovers and grasses (Table II)

The per cent N increased appreciably in grass and clover with applications of S, but was twice as high in the clover as in the grass. The per cent P was slightly higher in the clover than in the grass and both remained remarkably steady at all levels of S; this might be

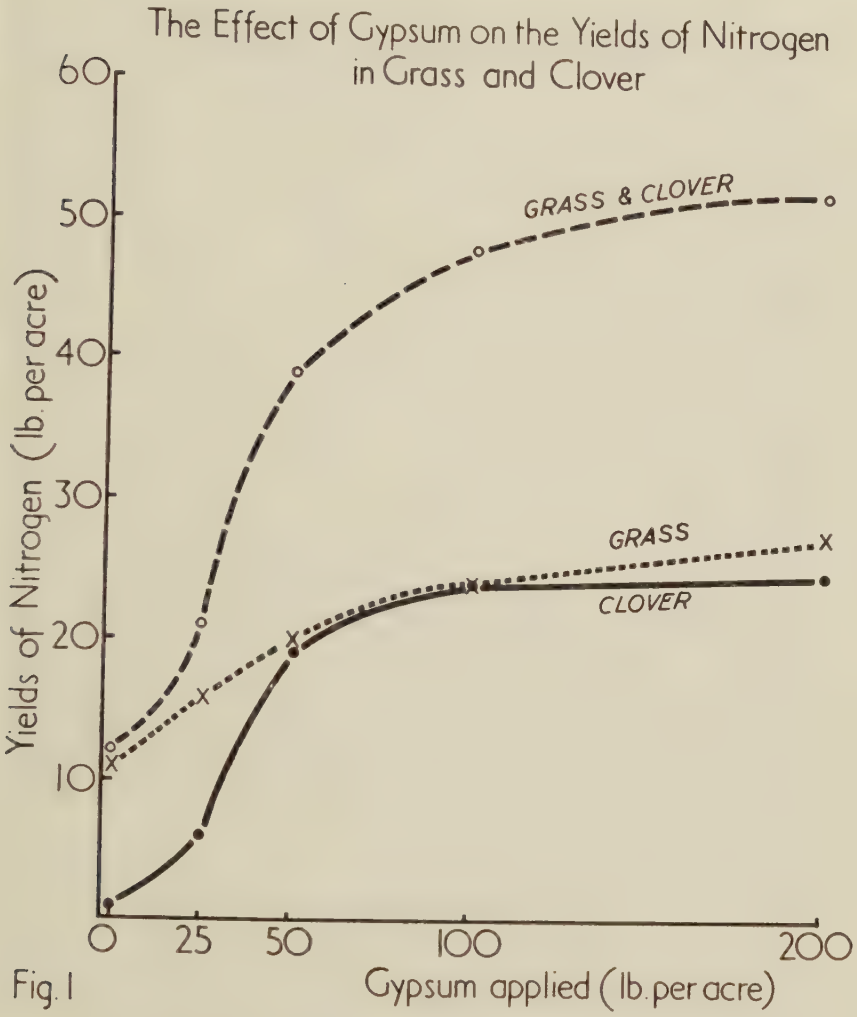

Fig. 1

expected in view of the very high P-status of the soil. Ca was typically higher in the clover than in the grass and increased slightly with S applications. On the other hand, Mn was as usual considerably higher in the grass and the content was little, if at all, affected by S. The total S content in the clover from the control treatment was low and increased appreciably with applications of S; inorganic S however was low throughout supporting previous observations [7]. Whilst

TABLE II

Composition of clover and grass (dry matter basis)					
Composition	Rates of gypsum				
	0	25	50	100	200
Clover					
N %	2.87	3.11	3.17	3.53	3.45
P %	0.29	0.26	0.28	0.25	0.24
Ca %	1.78	1.87	2.01	2.15	1.92
Mn ppm	118	123	123	119	136
Total S %	0.172	0.292	0.415	0.298	0.338
Inorganic S %	0.001	0.505	0.007	0.006	0.003
Grass					
N %	1.34	1.67	1.64	1.71	1.73
P %	0.23	0.22	0.23	0.21	0.24
Ca %	0.85	0.80	0.87	0.78	1.08
Mn ppm	332	338	333	337	273
Total S %	0.135	0.313	0.346	0.399	0.319
Inorganic S %	0.025	0.025	0.053	0.068	0.084

the per cent total S increased in the grass the inorganic S which was considerably higher than in the clover also increased markedly. It is surprising that the S contents were not higher at the high levels of application as dry matter yields did not increase appreciably at these levels. Considerably higher figures have been reported by Askew and Bishop[1] but stage of maturity may have been an important factor. It is of interest to compare the recovery of S with the amounts applied (Table III). The gypsum contained on analysis 20.9% S.

TABLE III

Recovery of S (lb per acre)			
S applied	Clover S	Grass S	Total S
0	0.07	1.10	1.17
5.2	0.57	2.88	3.45
10.5	2.52	4.26	6.78
20.9	2.01	5.63	7.64
41.8	2.39	5.08	7.47

A considerably bigger fraction of the S was taken up by the grass than by the clover, particularly under the deficiency conditions of the control and smallest application of S. The yield of 1.1 lb S in the control grass was almost a tenth of the yield of N (10.9 lb) supporting a suggestion made by Walker[5] that soil N and S may be

mineralised in roughly the same proportions as they occur in the soil organic matter. It would appear in this particular locality that very little S is obtained from the atmosphere because even as little as 5 lb S per acre markedly increased yields of dry matter and uptake of S, although less than half the applied S was recovered in the grass and clover tops. Just over 50 per cent of the 10.5 lb dressing of S was recovered in the tops, and considerably less at the higher rates. We have suggested elsewhere that the root yields of N in grass in a pasture may be about half the above-ground yields [9]. The amount for clover may be rather less than this (unpublished data), but assuming a top-root ratio of 2 : 1 of S for both grass and clover then 70–80% of the applied S would have been recovered at the two lowest rates of S and considerably less at the two highest rates.

Whilst lack of moisture over part of the growing season may have been responsible for the flattening of the response curve (dry matter and nitrogen), determination of the residual sulphate in the soil showed very little difference between the various treatments. The 0–4″ layer contained about 1.5 ppm SO_4–S in all cases, and as the soils were dried prior to analysis much of this could have some from further mineralisation of organic matter. The 4″–8″ layer contained about 1 ppm SO_4–S for the control and 2 ppm for the 100 and 200 lb rates of gypsum. These low figures for residual SO_4 were confirmed by measuring the specific conductance of the soils for a 1 : 5 soil: water ratio, obtaining an almost constant value of 2.2×10^{-5} mhos for all treatments at both depths. Clearly much of the SO_4 applied in the two heaviest dressings had been immobilised in the soil or lost by leaching*. Most of the rain recorded during the period fell in December and February and in one period of four consecutive days in December over $4\frac{1}{2}$ inches was recorded. The soil was coarse textured and freely drained and some of this rain must certainly have percolated through the soil. The solubility of gypsum is such (0.23 g per 100 ml) that 1″ of water saturated with gypsum could leach out more than 500 lb per acre. Gypsum may be leached very readily under high rainfall conditions, particularly if Mg and K replaced some of the Ca by cation exchange.

* Subsequent observations in the following spring indicate marked residual effects from the two higher dressings of gypsum. It is possible that SO_4 was absorbed as suggested by L. E. Ensminger (Proc. Am. Soc. Soil Sci. **18**, 259—264 (1954)) and not extracted by the 0.001 *N* HCl used in our work. This aspect is under examination.

DISCUSSION

This experiment has confirmed beyond reasonable doubt that clover responses in this area to applied gypsum are primarily sulphur responses and not associated with effects of calcium or other nutrient interactions. Responses may be obtained from the Ca in $CaSO_4$ on acid soils low in exchangeable Ca, and $CaSO_4$ may also increase uptake of Mn at least temporarily on some soils and by inference of such heavy metals as Cu, Zn and Co [10]. Indeed on acid soils high in exchangeable Mn toxic amounts of Mn due to applications of $CaSO_4$ may lead to poorer clover growth [7], an effect we have recently observed again (unpublished data).

The response of clovers is undoubtedly the primary effect but in previous trials [7] we were unable to examine the effects on grass because of the high experimental errors due to the uneven terrain and cover. The present experiment was sited on a uniform piece of soil with as even a cover of species as was possible, and in spite of the high experimental errors grass responded significantly to S treatments. Two possible mechanisms suggest themselves in explanation of this fact. Firstly, a direct response of the grass to the application of S, or secondly, an indirect effect on the grass resulting from increased N-fixation by the clovers and subsequent underground transference of N. If the grass had responded directly to S, the yield of N in the grass might have been expected to remain constant and the per cent N to fall as S applications increased. Actually the per cent N in the grass increased and the yield of N in the grass rose from 10.9 to 27.6 lb per acre. This extra nitrogen could have arisen from an increase in microbial activity and enhanced mineralisation of soil N due to the gypsum. This possibility was discounted by incubating soil from the control area with and without additions of gypsum (20 mg per 100 g soil) for one month at 30°C. The NH_4–N and NO_3–N were determined with the following results:

N compound	No S	S
NH_4–N ppm	45.8	49.7
NO_3–N ppm	7.9	6.9
Total mineral N ppm	53.7	56.6

These differences are within experimental error and it is therefore unlikely that gypsum increased the mineralisation of soil N in the

field. It seems highly probable therefore that the increased yields of grass resulted from underground transference of N from the clover, and as seen from Fig. 1 there is indeed a close correlation between the yields of N in the grass and clover. It has been suggested elsewhere [9] that under certain ill-defined optimal conditions clovers may excrete (or make available by other means) about half the N fixed symbiotically or about as much N as is retained in the aerial parts of the clover. When this N is utilised by associated grasses about two-thirds of it may be harvested in the grass tops, the rest going to increase root growth. It is of interest to note that the grass contained 16 lb more N for the 200 lb rate of gypsum than the control, which with the assumed increase in root N of 8 lb makes a total of 24 lb compared with the yield of 24 lb N in the clover tops. Under the particular conditions of this trial clovers appear to have excreted considerable amounts of N, relatively similar in extent to the amounts suggested by us in a theoretical consideration of the nitrogen economy of grass legume associations [9].

The total and inorganic S contents support earlier observations [7] that grasses may compete intensively with clovers for SO_4 to the point of luxury consumption. Negligible amounts of SO_4–S were present in the clovers and it was apparently rapidly utilised for amino-acid and protein syntheses.

There was no evidence in this soil that $CaSO_4$ may increase the Mn content of plants, which remained very constant throughout although total uptake of Mn increased greatly; the exchangeable Mn however was not high.

The evidence presented suggests that in this locality returns of S from the atmosphere during the season of active growth are exceptionally low. Even if the whole of the S harvested in the control plants is attributed to atmospheric sources it amounts only to about 1 lb per acre and this takes no account of mineralised soil organic S or the small amount of S supplied in the basal dressing of triple super (1.33% S). Sulphur responses have been reported over a wide area in this region [7] and it must be presumed that atmospheric returns are low throughout. It is usually assumed in well-drained soils in humid-temperate regions that most of the S is in the organic form with fairly constant N/S ratios. If S is mineralized at the same relative rate as N, grasses may utilise almost all the mineral N and S leaving clovers dependent on outside sources of S in order to fix N

symbiotically. Using N^{15} sources of mineral N on mixtures of grass and clover it has recently been shown that grass will utilise 95 per cent of the available N [8] and because of the capacity of grass to absorb luxury amounts of SO_4–S, fairly large amounts of S may be needed from sources other than the soil organic matter, if clovers are to fix much N. Even where atmospheric returns are low some parent materials may contain mineral S compounds and perhaps on shallow or recent soils derived from such materials inorganic S may be made available by weathering, an aspect worthy of study. In this same region we have had marked responses of clover and grass to gypsum on a rendzina containing 0.7 per cent N and 0.12 per cent S (unpublished data), supporting our claims that S accumulates along with N and other nutrients (particularly P) in soil organic matter, at least until the high equilibrium levels are reached, and that continual supplies of S are necessary if symbiotic N-fixation is to be maintained at a high level.

Our data suggest that leaching of applied gypsum may be fairly rapid as only small amounts of SO_4 were present down to a depth of 8″ on plots receiving 200 lb gypsum per acre in the spring. Hilder [3] showed in Australia that after annual applications of 2 cwt superphosphate per acre for four years the omission of superphosphate in the fifth year resulted in marked S-deficiency, and he concluded that the residual value of S provided as gypsum was small. Much must depend on the climatic conditions, soils, pasture cover and time of application. In the absence of legumes a certain amount of SO_4 will be taken up by the grass, but in the absence of fertiliser N growth of grasses will normally be restricted by dependence on the mineralisation of soil N and most of the sulphate applied as gypsum will be leached out. If legumes are present, SO_4–S will be needed to the extent that climatic conditions and management will permit optimum N-fixation, and S will accumulate in the soil organic matter. Under the conditions of this particular trial much of the SO_4 applied as gypsum may have been leached below 8″ during the growing season, and the flattening of the response curve may have been due not only to dry conditions in some months, but also in part to the loss of SO_4 by leaching at the high levels of application. This observation is supported to some extent by the failure of the S content of the herbage to increase with the heaviest applications; indeed the S contents of the grass and

clover were lower at the 200 lb rate than the 50 lb rate of gypsum.

From the practical point of view where atmospheric returns of S need to be supplemented by the use of S-containing fertilisers, there is everything to be said for applying such fertilisers as gypsum or superphosphate little and often. This is normally recommended in the case of superphosphate with the idea of reducing the extent of "P-fixation", and this practice may also reduce losses of SO_4 by leaching, particularly in regions of high summer rainfall. The practice in some parts of New Zealand of applying superphosphate in spring, and in late summer or early autumn when clovers will still grow for a further three months, may well be justified on this account alone. It is possible that other forms of S (*e.g.* elemental S) may be more efficient than gypsum if care is taken to avoid complications arising from its more pronounced effects on the acidity complex, and this comment is supported by experience in America, particularly in Oregon [4].

SUMMARY

1. Gypsum was applied at various levels to a grass-clover association, and its effect noted on the yields and composition of both components.

2. Both clover and grass responded markedly and a three-fold increase in dry matter and a four-fold increase in yield of N were noted.

3. The extra nitrogen in the grass was shown to have been derived almost certainly by underground transference from the clover, the amount involved approximating to the amount of N retained in the aerial part of the clover.

4. Whilst a high proportion of the S applied was recovered at rates up to 50 lb gypsum per acre, a low recovery was obtained from greater applications, and as residual SO_4 was very low, it is presumed that SO_4 was readily leached during one or two periods of heavy summer rainfall. The ease with which SO_4 is lost by leaching may merit attention to forms, times and rates of application of S.

5. Nearly all the S in the clover was organic, whereas a big fraction of the S in the grass was SO_4–S. In regions where atmospheric returns of S are small and where no S is being made available from the weathering of S-containing minerals, S must be applied in some form or other to promote optimum N-fixation by legumes as grass may utilise almost all the mineral N and S made available from soil organic matter.

6. Over large areas of the South Island of New Zealand it is doubtful if more than 1 lb S per acre is returned annually from the atmosphere and the application of S is of some considerable importance to promote N-fixation by clovers and hence high production from grass-clover associations.

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